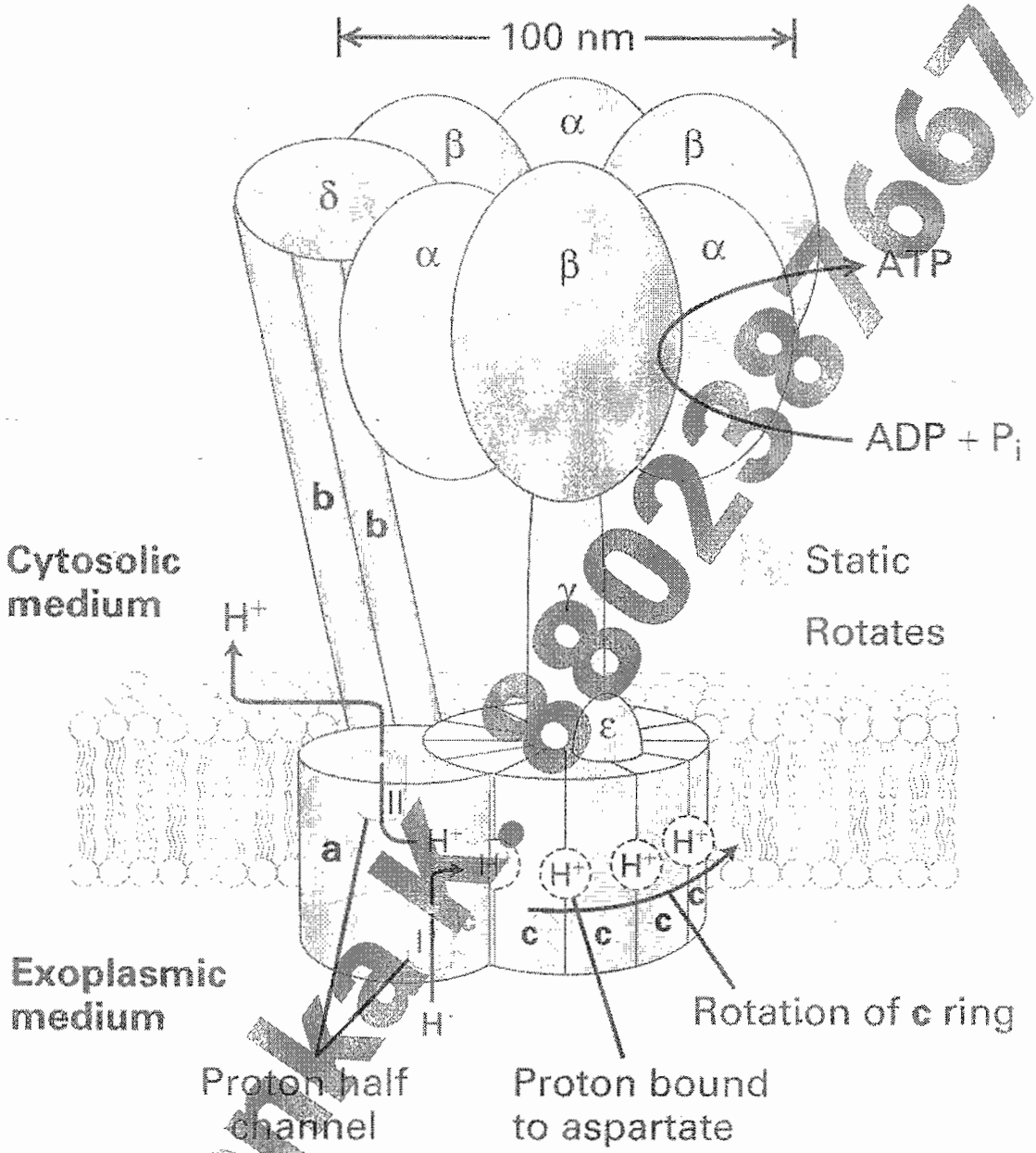
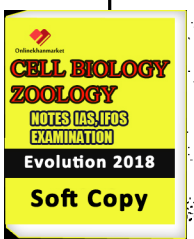


BOTANY

CELL BIOLOGY

TABLE OF CONTENTS



GENERAL INTRODUCTION TO A CELL

The Cell Theory

A cell is the smallest enclosed chemical system of open thermodynamic nature possessing a unique structural cohesion and showing functional self-sustainability, reproducibility, irritability and adaptiveness. It represents life at its smallest level – hence it is also called the **structural, functional**, and **reproductive unit of life**. The significance of the cell in this way was first put forward by the **Cell Theory**.

The Cell Theory, or Cell Doctrine, states that all organisms are composed of similar units of organization, called cells; and also that all cells arise from pre-existing cells.

In 1839, Theodor Schwann (a Zoologist) and Matthias Schleiden (a Botanist) contributed the earliest version of the Cell Theory. It summarized their observations into three conclusions about cells:

1. The cell is the unit of structure, physiology, and organization in living things.
2. The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.
3. Cells form by free-cell formation, similar to the formation of crystals (spontaneous generation).

We know today that the first two tenets are correct, but the third is clearly wrong. The correct interpretation of cell formation by division was finally promoted by others and formally enunciated in 1958 in Rudolph Virchow's dictum, "*Omnis cellula e cellula*", which means that all cells only arise from pre-existing cells.

The modern tenets of the Cell Theory include:

1. All known living things are made up of cells.
2. The cell is the fundamental unit of structure and function in living things.
3. Some organisms are unicellular, i.e., made up of only one cell.
4. Others are multicellular, composed of a number of cells.
5. All cells come from pre-existing cells by division.
6. Cells contain hereditary information (DNA) which is passed from cell to cell during cell division.
7. All cells are basically the same in chemical composition.
8. Energy flow (metabolism and biochemistry) occurs within cells.
9. The activity of an organism depends on the total activity of independent cells.

Exceptions to the Cell Theory

1. Viruses are considered by some to be alive because they show biological activity, yet they are not made up of cells.
2. The first cell did not originate from a pre-existing cell.

Organization of a plant cell

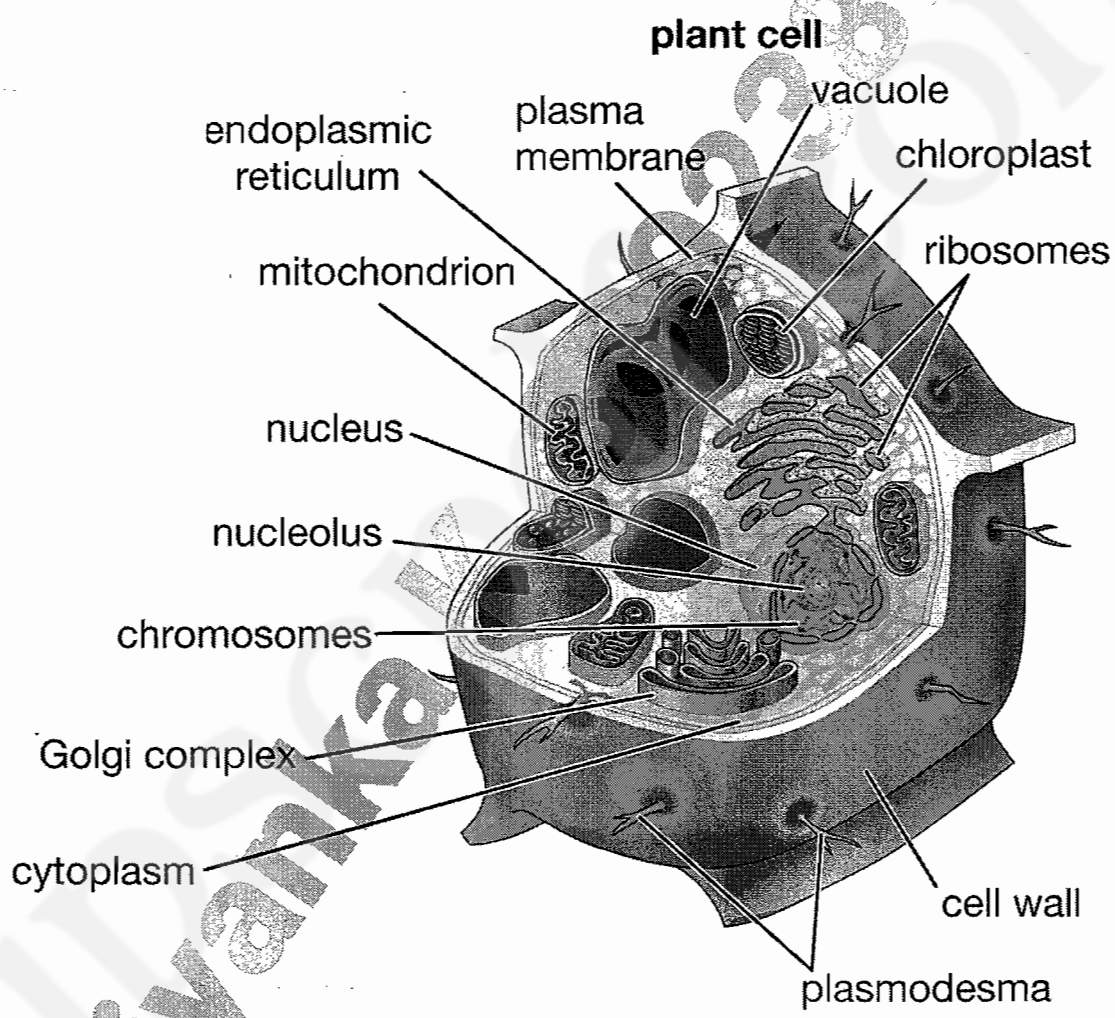


FIGURE 1: The structural orgaization of a plant cell.

A plant cell is a eukaryotic cell, showing the following features (Fig. 1).

1. Presence of a cell wall
2. Presence of plastids

3. The living part or the protoplasm delimited by a lipid bilayer membrane – called the Plasma Membrane
4. A central nuclear region encompassed by a delimiting double membrane, which contains the hereditary material.
5. Presence of all the structures required for biological processes, as shown in the table below.

***Table 1*: Structures in Plant Cells**

NAME	LOCATION	MAJOR FUNCTION
Cell wall	ECM	Protection, maintenance of shape etc.
Plasma membrane	Cell surface	Forming the boundary of the protoplasm, transport regulation
Cytoskeleton	Cytoplasm	Maintains cell shape, facilitates cell movement, cell division and movement of material within cell.
Cytosol	Cytoplasm	Protein rich fluid in which organelles, nuclear and other cytoplasmic components and cytoskeleton are immersed
Rough Endoplasmic Reticulum	Cytoplasm	Site of protein processing
Smooth Endoplasmic Reticulum	Cytoplasm	Lipid synthesis, Storage; Detoxification
Golgi complex	Cytoplasm	Processing, and sorting of proteins
Lysosomes	Cytoplasm	Digestion of imported material and cell's own used material
Peroxisomes	Cytoplasm	Metabolism of fatty acids and other metabolites; breaking down peroxides.
Plastids	Cytoplasm	Photosynthesis, starch storage, pigment storage
Mitochondria	Cytoplasm	Transform energy from food
Nucleus	Cytoplasm	Repository of the genetic material and early steps of gene expression
Nucleolus	Nucleus	Synthesis of rRNA and Ribosome Biogenesis
Ribosome	Cytoplasm (attached to Rough ER or Free Standing in the cytosol)	Site of protein synthesis

Introduction to various structures

Extracellular Matrix of Plant Cells

The plant cell wall is a specialized form of extracellular matrix that surrounds every cell of a plant and is responsible for many of the characteristics distinguishing plant from animal cells. Although often perceived as an inactive product serving mainly mechanical and structural purposes, the cell wall actually has a multitude of functions upon which plant life depends. Such functions include: (1) providing the protoplast, or living cell, with mechanical protection and a chemically buffered environment, (2) providing a porous medium for the circulation and distribution of water, minerals, and other small nutrient molecules, (3) providing rigid building blocks from which stable structures of higher order, such as leaves and stems, can be produced, and (4) providing a storage site of regulatory molecules that sense the presence of pathogenic microbes and control the development of tissues.

Plasma Membrane

The contents of the cell (**cytoplasm** and cytoplasmic organelles) are separated from the external surroundings by a limiting membrane (about 10 nm thick), known as the **plasma membrane** (also called **cell membrane** or **plasmalemma**), which is composed of lipid bilayer, embedded and superficially attached proteins, and trace amounts of conjugated carbohydrates. This structure regulates the passage of materials between the cell and its surroundings and in some tissues is involved in intercellular communication (e.g., nerve tissue).

The plasma membrane is not a homogeneous structure having the same chemical composition over its entire surface. Instead, the composition and organization vary in different regions of the membrane.

Cytoskeleton

The **cytoskeleton** (also known as CSK) is a cellular "scaffolding" or "skeleton" contained within the cytoplasm. The cytoskeleton is present in all cells; it was once thought this structure was unique to eukaryotes, but recent research has identified the prokaryotic cytoskeleton. It is a dynamic structure that maintains cell shape, often protects the cell, enables cellular motion (using structures such as flagella, cilia and lamellipodia), and plays important roles in both intracellular transport (the movement of vesicles and organelles, for example) and cellular division.

Eukaryotic cells contain three main kinds of cytoskeletal filaments, which are microfilaments, intermediate filaments, and microtubules.

Actin filaments / Microfilaments

Around 7 nm in diameter, this filament is composed of two intertwined actin chains. Microfilaments are most concentrated just beneath the cell membrane, and are responsible for resisting tension and maintaining cellular shape, forming cytoplasmatic protuberances (like pseudopodia and microvilli-although these by different mechanisms), and participation in some cell-to-cell or cell-to-matrix junctions. In association with these latter roles, microfilaments are essential to transduction. They are also important for cytokinesis (specifically, formation of the cleavage furrow) and, along with myosin, muscular contraction. Actin/Myosin interactions also help produce cytoplasmic streaming in most cells.

Intermediate Filaments

These filaments, 8 to 12 nanometers in diameter, are more stable (strongly bound) than actin filaments, and heterogeneous constituents of the cytoskeleton. Like actin filaments, they function in the maintenance of cell-shape by bearing tension (microtubules, by contrast, resist compression. It may be useful to think of micro- and intermediate filaments as cables, and of microtubules as cellular support beams). Intermediate filaments organize the internal tridimensional structure of the cell, anchoring organelles and serving as structural components of the nuclear lamina and sarcomeres. They also participate in some cell-cell and cell-matrix junctions.

*A fourth eukaryotic cytoskeletal element, **microtrabeculae**, was proposed by Keith Porter based on images obtained from high-voltage electron microscopy of whole cells in the 1970s. The images showed short, filamentous structures of unknown molecular composition associated with known cytoplasmic structures. Porter proposed that this microtrabecular structure represented a novel filamentous network distinct from microtubules, filamentous actin, or intermediate filaments. It is now generally accepted that microtrabeculae are nothing more than an artifact of certain types of fixation treatment though we have yet to fully understand the complexity of the cell's cytoskeleton.*

Different intermediate filaments are:

- made of vimentins, being the common structural support of many cells.
- made of keratin, found in skin cells, hair and nails.
- neurofilaments of neural cells.
- made of lamin, giving structural support to the nuclear envelope.

Microtubules

Microtubules are hollow cylinders about 25 nm in diameter (lumen = approximately 15nm in diameter), most commonly comprised of 13 protofilaments which, in turn, are polymers of alpha and beta tubulin. They have a very dynamic behaviour, binding GTP for polymerization. They are commonly organized by the centrosome.

In nine triplet sets (star-shaped), they form the centrioles, and in nine doublets oriented about two additional microtubules (wheel-shaped) they form cilia and flagella. The latter formation is commonly referred to as a "9+2" arrangement, wherein each doublet is connected to another by the protein dynein. As both flagella and cilia are structural components of the cell, and are maintained by microtubules, they can be considered part of the cytoskeleton.

They play key roles in:

- intracellular transport (associated with dyneins and kinesins, they transport organelles like mitochondria or vesicles).
- the axoneme of cilia and flagella.
- the mitotic spindle.
- synthesis of the cell wall in plants.

Endoplasmic Reticulum

The endoplasmic reticulum (ER) is a functionally versatile cell organelle, present as an anastomosing network of sacs (cisternae), tubules and vesicles enclosed by a continuous membrane, which extends from the nuclear envelope (NE) throughout the cytoplasm. The ER is in physical continuity with the nuclear envelope and shares an intimate functional relation with the Golgi Apparatus (GA). Together, the ER, GA and the NE make the *Endomembrane System* of a eukaryotic cell.

The ER serves as a multifunctional cell organelle. It serves specialized functions in the cell, including synthesis and modification of the proteins being targeted by the secretory pathway, organelle biogenesis, storage (and release) of calcium ions, production of steroids, lipid metabolism, storage and production of glycogen, etc.

The two morphological varieties of Endoplasmic Reticulum are called *Rough Endoplasmic Reticulum*, *Smooth Endoplasmic Reticulum*.

Rough Endoplasmic Reticulum (RER)

The surface of the rough endoplasmic reticulum is studded with protein-manufacturing ribosomes giving it a "rough" appearance (hence its name). However, these ribosomes are not resident of the endoplasmic reticulum initially. The ribosomes only bind to the ER once it begins to synthesize a protein destined for sorting via the rough ER.

The RER is key in producing:

- lysosomal enzymes with a Mannose-6-phosphate marker added in the cis-Golgi network
- Secreted proteins, either secreted constitutively with no tag, or regulated secretion involving clathrin and paired basic amino acids in the signal peptide.
- Integral membrane proteins that stay imbedded in the membrane as vesicles exit and bind to new membranes.

Smooth Endoplasmic Reticulum (SER)

The smooth endoplasmic reticulum has functions in several metabolic processes, including synthesis of lipids and steroids, metabolism of carbohydrates, regulation of calcium concentration, drug detoxification, attachment of receptors on cell membrane proteins, and steroid metabolism. It is connected to the nuclear envelope. Smooth endoplasmic reticulum is found in a variety of cell types (both animal and plant) and it serves different functions in each. The Smooth ER also contains the enzyme Glucose-6-phosphatase which converts glucose-6-phosphate to glucose, a step in gluconeogenesis. The Smooth ER consists of tubules and vesicles that branch forming a network. In some cells there are dilated areas like the sacs of rough endoplasmic reticulum. The network of smooth endoplasmic reticulum allows increased surface area for the action or storage of key enzymes and the products of these enzymes. The smooth endoplasmic reticulum is known for its storage of calcium ions in muscle cells.

Golgi Apparatus

The **Golgi Apparatus** (GA) is an organelle found as a part of the endomembrane system. The GA is organized as a complex composed of flat sacs or cisternae stacked parallel in a protein matrix, called the Golgi Matrix. The location of the GA is between the ER and the Plasma Membrane.

The Golgi is composed of membrane-bound stacks known as cisternae. Between five and eight are usually present. The cisternae stack has five functional regions: the cis-Golgi network, cis-Golgi, medial-Golgi, trans-Golgi, and trans-Golgi network. Vesicles from the endoplasmic reticulum (via the vesicular-tubular cluster) fuse with the cis-Golgi network and subsequently progress through the stack to the trans-Golgi network, where they are packaged and sent to the required destination. Each region contains different enzymes, which selectively modify the contents depending on where they are destined to reside.

The primary function of the Golgi apparatus is to process proteins targeted to the exoplasm, plasma membrane, lysosomes or endosomes. The Golgi apparatus is present in most eukaryotic cells, but tends to be more prominent where there are many substances, such as proteins, being secreted. For example, plasma B cells, the antibody-secreting cells of the immune system, have prominent Golgi complexes.

The Golgi Apparatus is the final processing and sorting centre for all those cellular proteins that are being targeted by the secretory pathway (see the diagram below). The GA processes these proteins and sort them within coated discharge vesicles. Most of the transport vesicles that leave the rough ER, are transported to the Golgi apparatus, where they are modified, sorted, and shipped towards their final destination. Thus, it functions as a protein delivery system for the cell.

Ribosome

Ribosomes are ribonucleoprotein complexes (made of RNA and protein) that are found in all cells. Ribosomes from animal cytoplasm are about 20nm in diameter and are composed of 65% ribosomal RNA and 35% ribosomal proteins. Eukaryotes have 80S ribosomes, each consisting of a small (40S) and large (60S) subunit. Their large subunit is composed of a 5S RNA (120 nucleotides), a 28S RNA (4700 nucleotides), a 5.8S subunit (160 nucleotides) and ~49 proteins. The 40S subunit has a 1900 nucleotide (18S) RNA and ~33 proteins.

In a eukaryotic cytoplasm there are two locations of ribosomes.

1. Free ribosomes are suspended in the cytosol (the semi-fluid portion of the cytoplasm)
2. Membrane attached ribosomes are bound to the rough endoplasmic reticulum, giving it the appearance of roughness and thus its name.

The function of ribosome is to translate messenger RNA (mRNA) to build polypeptide chains (e.g., proteins) using amino acids delivered by transfer RNA (tRNA). Their active sites are made of RNA, so ribosomes are now classified as "ribozymes."

Lysosomes

Lysosomes are organelles that contain low pH dependent digestive enzymes (acid hydrolases). They digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane surrounding a lysosome allows the digestive enzymes to work at the 4.5 pH they require.

Some important enzymes in lysosomes are:

- Lipase, which digests lipids
- Carbohydrases, which digest carbohydrates (e.g., sugars)

- Proteases, which digest proteins
- Nucleases, which digest nucleic acids
- phosphoric acid monoesters.

The lysosomes are used for the digestion of macromolecules from phagocytosis (ingestion of other dying cells or larger extracellular material), endocytosis (where receptor proteins are recycled from the cell surface), and autophagy (wherein old or unneeded organelles or proteins, or microbes that have invaded the cytoplasm are delivered to the lysosome). Autophagy may also lead to autophagic cell death, a form of programmed self-destruction, or autolysis, of the cell, which means that the cell is digesting itself.

Other functions include digesting foreign bacteria (or other forms of waste) that invade a cell and helping repair damage to the plasma membrane by serving as a membrane patch, sealing the wound. In the past, lysosomes were thought to kill cells that were no longer wanted, such as those in the tails of tadpoles or in the web from the fingers of a 3- to 6-month-old fetus. While lysosomes digest some materials in this process, it is actually accomplished through programmed cell death, called apoptosis.

Peroxisomes

Peroxisomes are microbodies that participate in the metabolism of fatty acids and other metabolites. Peroxisomes have enzymes that rid the cell of toxic peroxides. They have a single lipid bilayer membrane that separates their contents from the cytosol (the internal fluid of the cell) and contain membrane proteins critical for various functions, such as importing proteins into the organelles and aiding in proliferation.

Mitochondria

A mitochondrion (plural: *mitochondria*) is a membrane-enclosed organelle found in most eukaryotic cells. These organelles range from 1–10 micrometers (μm) in size. Mitochondria are sometimes described as "cellular power plants" because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth.

The organelle is composed of compartments that carry out specialized functions. These compartments or regions include the outer membrane, the intermembrane space, the inner membrane, and the cristae and matrix.

The outer mitochondrial membrane, which encloses the entire organelle, has a protein-to-phospholipid ratio similar to that of the eukaryotic plasma membrane (about 1:1 by weight). It contains large numbers of integral proteins called *porins*. These porins form channels that allow molecules 5000 Daltons or less in molecular weight to freely diffuse from one side of the membrane to the other.

The intermembrane space is basically the space between the outer membrane and the inner membrane. Because the outer membrane is freely permeable to small molecules, the concentrations of small molecules such as ions and sugars in the intermembrane space is the same as the cytosol.

The inner mitochondrial membrane contains proteins with four types of functions:

1. Those that perform the redox reactions of oxidative phosphorylation

2. ATP synthase, which generates ATP in the matrix
3. Specific transport proteins that regulate metabolite passage into and out of the matrix
4. Protein import machinery.

It contains more than 100 different polypeptides, and has a very high protein-to-phospholipid ratio (more than 3:1 by weight, which is about 1 protein for 15 phospholipids). The inner membrane is home to around 1/5 of the total protein in a mitochondrion.

The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total protein in a mitochondrion. The matrix is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane. The matrix contains a highly-concentrated mixture of hundreds of enzymes, special mitochondrial ribosomes, tRNA, and several copies of the mitochondrial DNA genome. Of the enzymes, the major functions include oxidation of pyruvate and fatty acids, and the citric acid cycle.

Nucleus

The nucleus is an enveloped cellular compartment of all eukaryotic cells except some specialized ones, visible only during the interphase of the cell cycle, which contains the genomic DNA and serves as the site of initial stages of gene expression. Since all the types of genome expression begin in the nucleus, which ultimately govern the entire range of cellular functions – it is also commonly referred to as **the control centre of the cell**.

The shape is mostly oval or spherical but it can vary. In most cells, the nucleus is spherical or oblong, which minimizes the surface area needed to enclose a specific volume. The percentage of total cell volume occupied by nuclei of different types of cells varies widely, from 1–2 percent in yeast cells, to 10 percent in most somatic cells, to as much as 40–60 percent in cells that have less need for cytoplasmic functions such as secretion.

A combination of Electron Microscopy and sophisticated biochemical analysis reveals that the nucleus has the following components:

1. The **nuclear envelope** has two concentric membranes (inner and outer nuclear membranes) that surround the nucleus and its underlying intermediate filament lattice, the **nuclear lamina**. The nuclear envelope is penetrated by nuclear **pores**. The outer membrane is continuous with the membrane of the rough endoplasmic reticulum. A **nuclear pore complex (NPC)** is a very large, proteinaceous structure that extends through the nuclear envelope, providing a channel for bidirectional transport of molecules and macromolecules between the nucleus and the cytosol.
2. The **nucleolus** (*plural:* nucleoli) is a discrete region of the nucleus where ribosomes are produced.
3. **Nuclear matrix** which is a filamentous network to which the chromatin material attach.
4. The **nucleoplasm** refers to the content of the nucleus, excluding the nucleolus. The nucleoplasm contains **Replication Factories** and **Transcription Factories** [Scheer *et al*, 2004]
5. **Heterochromatin** describes regions of the genome that are highly condensed, are not transcribed, and are late-replicating. Heterochromatin is divided into two types, which are called constitutive and facultative.

6. **Euchromatin** comprises all of the genome in the interphase nucleus except for the heterochromatin. The euchromatin is less tightly coiled than heterochromatin, and contains the active or potentially active genes.

PROKARYOTIC CELLS

Note: For a tratement of prokaryotic cells in greater depth, please refer to your MICROBIOLOGY Notes.

The prokaryotes are the simplest type of cellular organisms. They are characterized by an absence of nucleus. They also do not have membrane bound organelles.

Salient features of the prokaryotes are as follows:

- Simplest living organisms, all of them lack nucleus around the cellular genome
- Include members from the two most primitive domains of life:
    1. Domain Bacteria
    2. Domain Archaea

A prokaryotic cell: Characteristic features

- Small cell size (0.5 μm to 10 μm of diameter)
- Mycoplasmas are the smallest prokaryotes, measuring 0.1 to 0.2 μm in diameter.
- Always unicellular, either solitary or colonial
- Prokaryotes have a nucleoid (nuclear body) rather than an enveloped nucleus and lack membrane-bound cytoplasmic organelles.
- The plasma membrane in prokaryotes performs many of the functions carried out by membranous organelles in eukaryotes.
- Multiplication is by binary fission.

Surface Structures

- Flagella: The flagella of motile bacteria differ in structure from eukaryotic flagella. A basal body anchored in the plasma membrane and cell wall gives rise to a cylindrical protein filament. The flagellum moves by whirling about its long axis. The number and arrangement of flagella on the cell are diagnostically useful.
- Pili (Fimbriae): Pili are slender, hairlike, proteinaceous appendages on the surface of many (particularly Gram-negative) bacteria. They are important in adhesion to host surfaces.
- Capsules: Some bacteria form a thick outer capsule of high-molecular-weight, viscous polysaccharide gel; others have more amorphous slime layers. Capsules confer resistance to phagocytosis.

- Cell Wall: It is a rigid structure made of peptidoglycans, which confer the characteristic cell shape and provide the cell with mechanical protection. Peptidoglycans are unique to prokaryotic organisms. These walls also contain Teichoic Acids and Lipoteichoic Acids.
- Two groups of bacteria are devoid of cell wall. They are:
    - the Mycoplasma species
    - the L-forms that arise from either Gram-positive or Gram-negative bacterial cells that have lost their ability to produce the peptidoglycan structures.
- The outer membrane of Gram-negative bacteria: It is composed of lipopolysaccharides.
- Plasma Membrane: The bacterial plasma membrane is composed primarily of protein and phospholipid (about 3:1). It performs many functions, including transport, biosynthesis, and energy transduction.

Cytoplasmic structures

- The bacterial cytoplasm is densely packed with 70S ribosomes.
- Other granules represent metabolic reserves (e.g., poly-β-hydroxybutyrate, polysaccharide, polymetaphosphate, and metachromatic granules).
- Endospores: Bacillus and Clostridium species can produce endospores: heat-resistant, dehydrated resting cells that are formed intracellularly and contain a genome and all essential metabolic machinery. The endospore is protected in a complex protective spore coat.

Nucleoid

- The bacterial nucleoid, which contains the DNA fibrils, lacks a limiting membrane.
- The bacterial nucleoid is a structure containing a single circular DNA without any histone protein.
- It is about 4-8 Mbp large and with a molecular weight of approximately 3x109. The unfolded DNA is about 1 mm long.

Difference between a prokaryotic cell and a eukaryotic cell

Characteristic	Prokaryotes	Eukaryotes
Size of cell	Typically 0.2-2.0 m m in diameter	Typically 10-100 m m in diameter
Nucleus	No nuclear membrane or nucleoli (nucleoid)	True nucleus, consisting of nuclear membrane & nucleoli
Membrane-enclosed organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria & chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple
Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors present
Cytoplasm	No cytosketeton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA) arrangement	Single circular chromosome; lacks histones	Multiple linear chromosomes with histones
Cell division	Binary fission	Mitosis
Sexual reproduction	No meiosis; transfer of DNA fragments only (conjugation)	Involves Meiosis

PLANT CELL E.C.M. — CELL WALL

INTRODUCTION TO ECM

The Extra Cellular Matrix or ECM is a non-living exoplasmic structure, secreted and patterned just outside the Plasma Membrane by the protoplasm of the cell, which plays essential roles in cellular physiology, defence and maintaining the cell in its social context. Despite being non living, the ECM of living cells are not inert 'boxes' but complex and dynamic sub-cellular compartments that play diverse and subtle roles in growth, development and defence.

Most of the cells, prokaryotic or eukaryotic, have ECM. The chemical nature and organization of ECM change with the individual cell type. For example:

1. The Eubacteria have peptidoglycan cell wall.
2. Plants (Bryophytes, Pteridophytes, Gymnosperms & Angiosperms) and charophycean alage have predominantly cellulosic cell walls.
3. Non-charophycean algae have different types of cellulosic walls with unique matrix compounds like alginic acid.
4. Fungi have chitinous cell wall.
5. Animal cells often have various types of cell junctions etc.
6. Several protozoans have a predominantly proteinaceous pellicle.

The cell wall of plants and charophycean algae is a strong, often rigid, extra-protoplasmic structure with highly organized composite formation with various polysaccharides (most notably celluloses, hemicelluloses, and pectins), proteins, aromatic compounds and various ions. The formation, growth and dynamics of the cell wall is directed from within the cell. Cell walls provide a skeletal support to the whole plant and also a barrier against injury and infection.

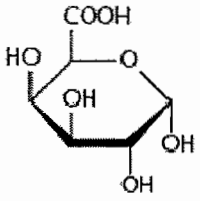
The cell wall of prokaryotes is a rather rigid structure that lies inside the capsular layer, but outside the plasma membrane of cells. **In cyanobacteria** it is composed primarily of cellulose, but in bacteria it is composed of a mixture of materials not found elsewhere. **Gram-positive bacteria** have relatively thick walls (15-80 nm) composed principally of peptidoglycans, also known as Murein (40-90 per cent), techoic acids, and other complex polysaccharides, with no differentiation into separate layers. **Gram-negative bacteria** have much thinner walls (10 nm) composed of several distinct layers which are chemically different from those of Gram-positive species. Peptidoglycans represent only 1-10% percent of the wall material by weight; techoic acids are absent, but complex lipopolysaccharides are present.

Chemical Composition

Chemically, the plant cell wall is composed of **water** [up to 70 %, by weight *in vivo*]. The dry matter is typically ~90% Polysaccharide and ~10% (glyco)protein. Classically, the wall is fractionated into three polysaccharide classes: pectins, hemicelluloses and cellulose. Moreover, there is a variable (but smaller) amount of **lipids**, **lignins**, **tannins**, and **mineral salts**. Various components of the cell wall exhibit distinct staining reactions as shown in the table below.

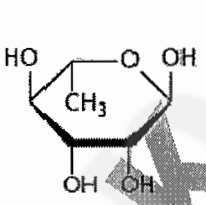
	Substance	Chemical unit	Staining reaction
1.	Cellulose	Glucose	Chlorzinc iodide (stains violet)
2.	Hemicellulose	Arabinose, xylose, mannose, glucose and galactose	No specific stain
3.	Pectin	Glucuronic and galacturonic acid	Ruthenium red
4.	Lignin	Coniferyl alcohol (*e.g.*, hydroxy-phenyl propane)	Phloroglucinol hydrochloride (stains rose); chlorzinc iodide (stains yellow)
5.	Cuticular substances	Fatty acids	Sudan III (stains orange)
6.	Mineral deposits	Calcium and magnesium as carbonates and silicates	

The diagrams below & table on the next two pages summarizes the chemical nature of various wall polysaccharides.



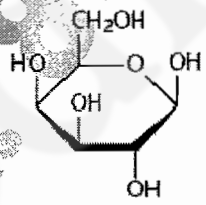
[I]

α-D-Galacturonic acid



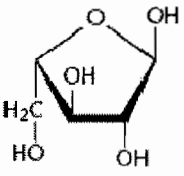
[II]

α-L-Rhamnose



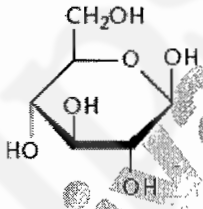
[III]

β-D-Galactose



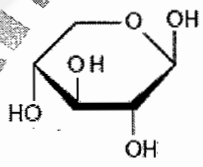
[IV]

α-L-Arabinose (furanose form)



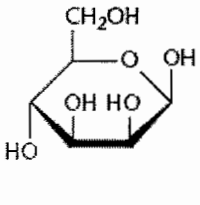
[V]

β-D-Glucose



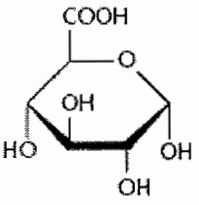
[VI]

β-D-Xylose



[VII]

β-D-Mannose



[VIII]

α-D-Glucuronic acid

Table 1 Polysaccharides and structural proteins of primary cell walls

Polymer	Amount (%)[a]	Q^b	Principal building blocks	Notes
Polysaccharides				
Microfibrillar				
Cellulose	30	0	βGlc	Linear (1→4)-Glc$_n$. Hydrogen-bonded within a microfibril
Matrix				
Pectins				
Homogalacturonan	16	–	αGalA	Linear (1→4)-GalA$_n$. Some of GalA residues methylesterified, some *O*-acetylated. Nonesterified homogalacturonan is hydrolysed by EPG
Rhamnogalacturonan-I	10	–	αGalA, αRha, βGal, αAra*f*, ...	Backbone [-GalA-(1→4)-Rha-(1→2)-]$_n$. ~Half the Rha residues carry Gal/Ara*f*-rich oligomer side-chains. GalA residues *O*-acetylated. Not hydrolysed by EPG
Rhamnogalacturonan-II	4	–	α&βGalA, α&βRha, αGal, αFuc, αAra*p*, βAra*f*, βApi*f*, βGlcA, KDO, βAce*f*A, αXyl, βDHA...	Extraordinarily complex polymer. α-(1→4)-GalA-rich core. Other residues as oligosaccharide side-chains attached via Api and KDO residues. Some Fuc and all Xyl residues as 2-*O*-methyl ethers. Some Api*f* residues esterified with borate. Not hydrolysed by EGP
Apiogalacturonan	±	–	αGalA, Api*f*, ...	Only in certain aquatic angiosperms e.g. *Lemna*
Hemicelluloses				
Xyloglucans	20	0	βGlc, αXyl, βGal, αFuc (±αAra*f*, βXyl)	Backbone (1→4)-Glc$_n$. Frequent repeat units include XXXG and XXFG where X = αXyl-(1→6)-βGlc*, G = βGlc*, F = αFuc-(1→2)-βGal-(1→2)-αXyl-(1→6)-βGlc*; asterisked residues = part of backbone. Some Gal residues *O*-acetylated
Xylans	8	–	βXyl, αAra*f*, α-GlcA, β-D-Gal (± L-Gal, ...)	Backbone (1→4)-Xyl$_n$. Side-chains, linked to C-2 or C-3 of backbone, include Ara*f*, GlcA, Xyl-(1→2)-Ara*f* and longer oligosaccharides. Some Xyl *O*-acetylated. Some Ara*f* residues have ferulate esterified to C-5
Mannans	±	0	βMan, βGlc, αGal	Little studied in primary cell walls. Well known in secondary walls of xylem and in some seeds
Mixed-linkage glucans (MLGs)	±	0	βGlc	Only in graminaceous monocots. Linear polymer with ~70% (1→4), 30% (1→3)-linkages
Callose	±	0	βGlc	Linear, (1→3)-Glc$_n$
Glucuronomannans	±	–	βGlcA, αMan, Ara*f*, Xyl, Gal	Backbone (-Man-(1→4)-GlcA-(1→2)-)$_n$. Ara*f*, Xyl and Gal residues as side-chains

continued

Table 1 *continued*

Polymer	Amount (%)[a]	Q^b	Principal building blocks	Notes
Proteins				
Extensins	±	+	Hyp, Ser, Lys, Tyr. Val. His: 50–60% sugar [β&αAra*f*, αGal]	Basic polypeptide backbone. Tetrasaccharide (αAra*f*-(1→3)-βAra*f*-(1→2)-βAra*f*-(1→2)-βAra*f*-) *O*-linked to most Hyp residues. Single Gal attached to some Ser residues. Some Tyr coupled to form isodityrosine
Arabinogalactan proteins (AGPs)	±	–	Hyp, Ser, Asp, Thr, Gly; 90–98% sugar [βGal, αAra*f*, GlcA, ...]	Slimes, e.g. gum arabic. Short, acidic polypeptide backbone. Polysaccharide groups (rich in (1→3) & (1→6)-linked Gal) *O*-linked to Hyp. Some AGPs tissue-specific. Some covalently attached to lipids
Proline-rich proteins (PRPs)	±	+	Pro, Hyp, Val, Tyr, Lys	Hyp:Pro ratio ~1:1. May become covalently crosslinked, possibly via Tyr residues. Little or no sugar. Sometimes associated with lignin.
Glycine-rich proteins (GRPs)	±		Gly; also Ser, Ala, ...	Often 60–70% Gly. Not glycosylated. Often associated with lignin

[a]Rough guide to amount of polymer present, as % of dry weight of a typical dicot primary cell wall from a rapidly growing cell culture. ±, not always present; –, amount varies greatly.
[b]Charge on polymer molecule (at physiological pH): –, negative; +, positive; 0, uncharged.
AceA, L-acerate; Ala, L-alanine, Api, D-apiose; Ara, L-arabinose; Asp, L-aspartate; D, optical isomer: DHA, 3-deoxy-2-D-heptulosarate; DP, degree of polymerization; EPG, endo-polygalacturonase; *f*, furanose ring-form; Fuc, L-fucose; Gal, galactose (D unless otherwise stated); GalA, D-galacturonate; Glc, D-glucose; GlcA, D-glucuronate; Gly, glycine; His, L-histidine; Hyp, L-hydroxyproline; KDO, D-ketodeoxyoctulosonate; L, optical isomer; Lys, L-lysine; Man, D-mannose; *O*-, via oxygen atom; Pro, L-proline, Rha, L-rhamnose; Ser, L-serine; Thr, L-threonine; Tyr, L-tyrosine; Val, L-valine, Xyl, D-xylose. All sugar residues are in the pyranose (*p*) ring-form unless indicated *f*.

1. **Cellulose** is a linear, unbranched polymer, consisting of straight polysaccharide chains made of about 15000 glucose units linked by 1-4 β glycosidic bonds. Cellulose is the principal fibrillar component of the plant cell walls, accounting for about 9 – 25% of the dry weight of primary walls and upto 50% of the dry matter of secondary walls. Cellulose chains produce the structural units known as **microfibrils** by inter-molecular hydrogen bondings. Each microfibril is composed of about 2000 cellulose chains in it and is 10 nm wide and 3 nm thick. At any sectional plane a microfibril is between 36 – 48 cellulose chain thick.

   Often between 4 and 7 microfibrils get associated to form the **macrofibrils** having up to 30nm diameter. It is the macrofibrils which interact with hemicelluloses. (Several authors still loosely use the terms microfibrils and macrofibrils interchangeably).

2. **Hemicelluloses** are short but **branched heteropolymers** of various kinds of monosaccharides such as arabinose, xylose, mannose, galactose, glucose and uronic acid. Some of the common hemicelluloses go under the names xylans, arabinoxylans, glucomannans, galactomannans and xyloglucans. The hemicelluloses account for about 25-50% of primary wall dry weight and about 30% in secondary walls. **In 1980s, Nicholas Carpita** showed that we can identify two major classes of cell walls in the plant world on the basis of the predominant hemicellulosic composition.

   a. **Type I cell wall:** found in all theBryophytes, Pteridophytes except the lycophytes, Gymnosperms and All the dicot Angiosperms plus nearly half of the monocots as well. In this type of cell wall **Xyloglucans (Xyg)** is the major hemicellulose. Xyg is a linear chain of 1-4 β D-Glucan with

xylose projections at frequent intervals. The xylose projections at frequent intervals provide a stable cross linking.

b. **Type II cell wall:** found in the lycophytes and the commelinoid monocots, such as Grasses, Cypercaceae members, Ginger, Palm etc. in this type of wall, **Glucuronoarabinoxylans (Gax)** is the major hemicellulose. The cross linking provided by Gax is not very stable in comparison to Xyg. Gax is a linear chain of 1-4 β xylose with occasional projections of arabinose and glucuronic acid.

3. **Pectins** are water soluble, heterogeneous branched polysaccharides that contain many negatively charged D-galacturonic acid residues along with D-glucuronic acid residues. Because of their negative charge, pectins are highly hydrated and intensely bind cations. When Ca^{2+} is added to a solution of pectin molecules, it cross-links them to produce a semirigid gel. Such Ca^{2+} cross-links are help to hold cell-wall components together. Important pectins are Homogalactouronan and Rhamnogalactouronan (Type I and Type II).

4. **Callose** is not a regular wall polysaccharide but produced in large amounts under special conditions. Callose is a short chain length β 1-3 Glucan Polymer. Callose does not cross link by the mediation of hemicelluloses and it can form a solid wall more quickly. Callose deposition is seen in growing pollen tube, wounding conditions, cell plate formation etc.

5. In addition, **Agar** is a polysaccharide, found in the cell wall of sea weeds and containing D-and L-galactose residues.

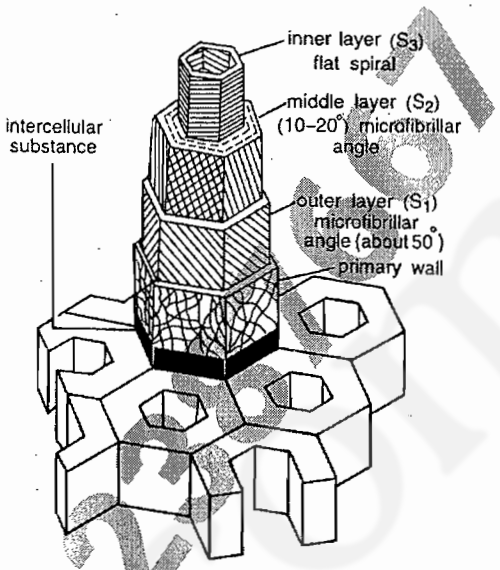
6. **Lignin** is a biological plastic and non-fibrous material. It occurs only in mature cell walls and is made of an insoluble hydrophobic aromatic polymer of phenolic alcohols *(e.g.,* hydroxyphenyl propane).

7. **Glycoproteins** (present up to 10 per cent in primary cell wall) are hydroxyproline rich proteins (like the collagen). These glycoproteins are known to act like the glue to increase the strength of the cell wall and also help in keeping the cell wall firmly attached to the plasma maembrane.

8. **Waxy materials: Cutin** is also a biological plastic and is made of fatty acids (waxes). **Suberin** is a water-resistant substance, comprising of fatty acids and found in the cork and cell wall of many plants. **Sporopollenin** is a lipoidal polymer forming tough wall with species-specific patterns of pollen grains.

9. **Mineral deposits** occur in cuticle in the form of calcium and magnesium carbonates and silicates. Deposits of calcium compounds are found in the cell wall of cruciferous and cucurbitaceous plants. Silicate deposits are common in the cell wall of Graminae family.

FINE STRUCTURE



The cell wall is complex in nature and is differentiated in the following layers (1) Primary cell wall; (2) Secondary cell wall; (3) Tertiary cell wall.

(1) **Primary cell wall** is the first formed & the outermost layer of the cell wall. in the young meristematic and parenchymatous cells it is the only cell wall. It is comparatively thin and permeable. Certain epidermal cells of the leaf and the stem also possess the cutin and cutin-waxes which make the primary cell wall relatively impermeable.

(2) **Secondary cell wall** follows the primary cell wall. It is thick, permeable and lies near the plasma membrane. It is composed of three concentric layers (S-1 S-2 and S-3) which occur one after another. Chemically the secondary cell wall is composed of compactly arranged **macrofibrils of the celluloses**, in between which sometimes occurs lignin as a inter-fibrillar material.

(3) **Tertiary cell wall** develops only in certain plant cells. It is an additional wall beneath the secondary wall. The tertiary cell wall differs from the primary and secondary cell wall in its morphology, chemistry and staining properties. Besides the cellulose, the tertiary cell wall consists of **xylan** and **lignin**.

Middle lamella is the inter-cellular matrix where the cells of plant tissues generally remain cemented together. The middle lamella is mainly composed of the **pectin, lignin and some proteins**.

MOLECULAR STRUCTURE

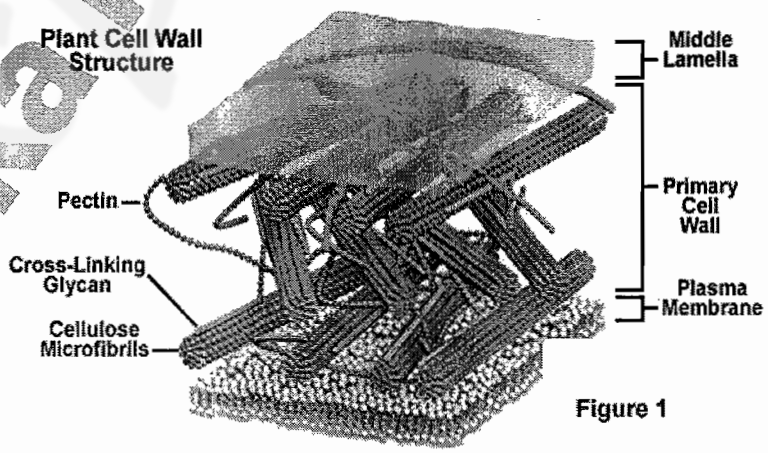


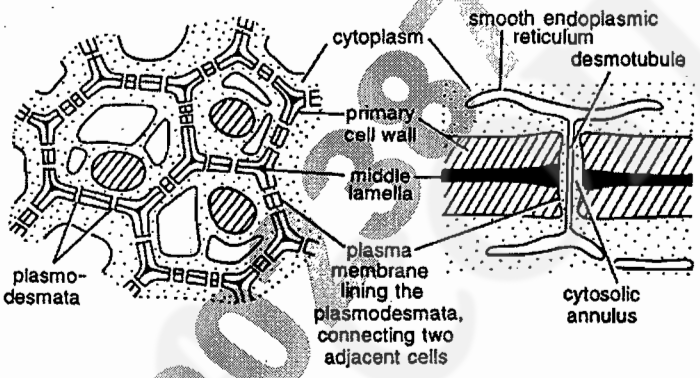
Figure 1

Electron microscopy has shown that the cell wall is constructed on similar architectural principle which applied well in the construction of animal bones or reinforced concrete (concrete + metal framework), *i.e.*, **strong fibres (*e.g.*, cellulose microfibrils) resistant to tension embedded in an amorphous matrix (comprising hemicellulose, pectin and proteins) resistant to compression**. In the primary cell wall, the fibres and matrix molecules are cross-linked by a combination of covalent bonds and non-covalent bonds to form a highly complex structure whose composition is generally cell-specific. In fact, hemicellulose molecules (*e.g.*, xyloglucans) are linked by hydrogen bonds to the surface of the cellulose microfibrils. Some of these hemicellulose molecules are cross-linked in turn to acidic pectin

molecules. through short neutral pectin molecules (*e.g.*, arabinogalactans). Cell wall glycoproteins are tightly woven into the texture of the wall to complete the structure of matrix.

In the multilamellar secondary cell wall, cellulose microfibrils are laid down in layers, the microfibrils of each layer running roughly parallel with each other but at an angle to those in other layers.

PLASMODESMATA



Every living cell in a higher plant is connected to its living neighhours by fine cytoplasmic channels, known as plasmodesma, which pass through the intervening cell walls. The plasma membrane & ER of one cell is continuous with that of its neighbour at each plasmodesma. A plasmodesmata is a roughly cylindrical, membrane-lined channel with a diameter of 20 to 40 nm. Running from cell to cell through the centre of most plasmodesmata is a narrower cylindrical structure, the **desmotubule, which is actually the continuum of ER.**

Since a plasmodesmata pierce the cell wall and connect all cells in the plant other cell wall, they function in intercellular communication forming the symplast, i.e. they allow molecules to pass directly from one cell to another. So, plasmodesmata are especially abundant in the walls of columns of cells that lead toward sites of intense secretion, such as in nectar secreting glands (trichomes of *Abutilon* nectaries).

Experimental evidence has suggested that they allow the passage of molecules with m.w. of less than 800 Daltons. Transport through the plasmodesmata is also found under complex regulations which may involve $Ca2^{+}$ and protein phosphorylation. Certain plant viruses such as TMV can enlarge plasmodesmata in order to use this route to pass from cell to cell. Tobacco mosaic virus is known to synthesize a protein, called P^{30}(30,OOO dalton M. W.) that nullifies the normal regulatory mechanisms of plasmodesmata.

CELL PLATE FORMATION DURING PLANT CELL MITOSIS

During Mitosis in plants, the daughter chromosomes separate during anaphase and migrate toward their respective poles. In contrast to animal cells, the spindle poles of plant cells is quite diffuse, so that the chromosomes move in nearly parallel fashion. At the end of teleophase, cytokinesis begins with the formation of the cell plate. This process involves fusion of many small secretory vesicles, and attachment of the resulting structure to the plasma membrane.

A diagram of the steps involved in cell plate formation is shown below:

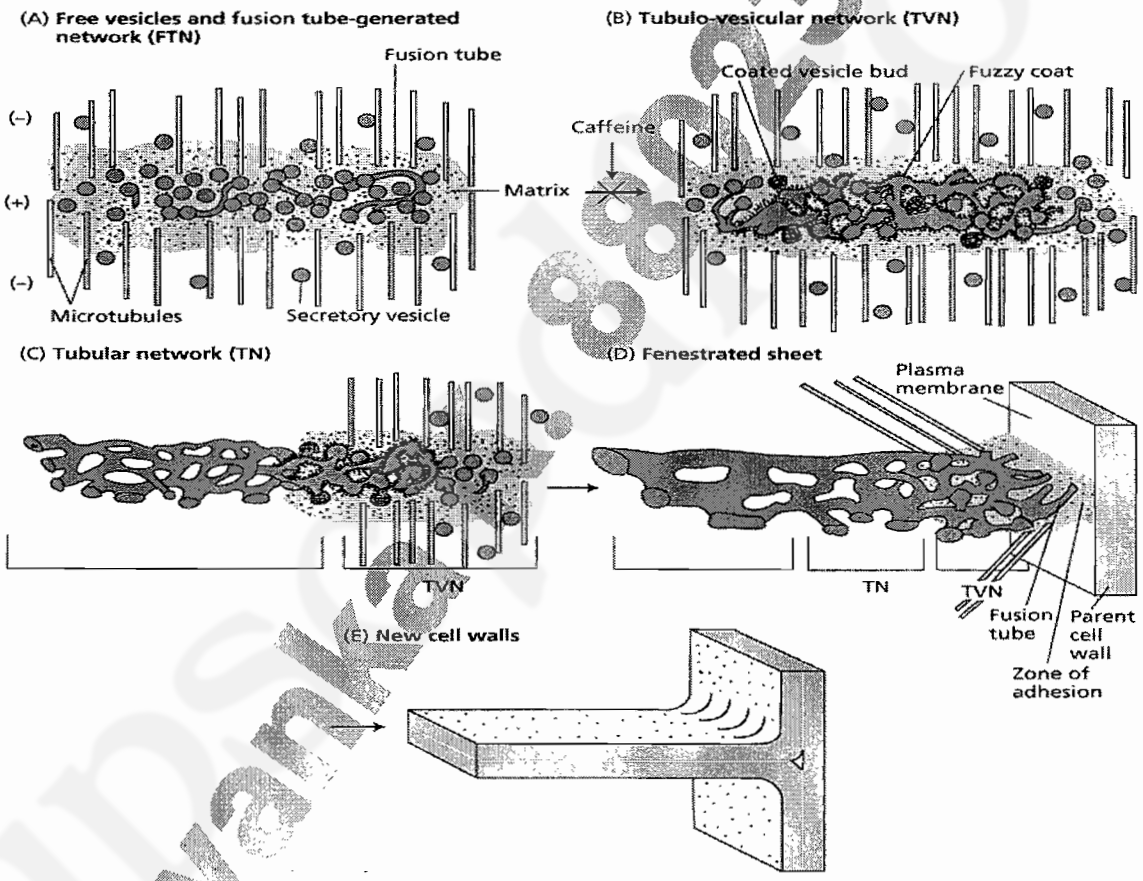
(A) In the first step, Golgi vesicles, some of which are interconnected via *fusion tubes,* aggregate in the spindle midzone area. This structure is called the fusion tube network (FTN). The transition from the first to the second stage of cell plate formation can be inhibited by caffeine.

(B) Formation of a tubulo-vesicular network (TVN). The contents of the vesicles, mainly pectins, represent the precursors from which the new middle lamella is assembled outside the cell. In the next stage, vesicle fusion increases, forming a tubulo-vesicular network (TVN), and the membranes become coated with either clathrin or other proteins.

(C) In the third stage, the central region of the growing cell plate forms a tubular network (TN), with vesicle fusion occurring at the growing edges where the remaining microtubules are located.

(D) In the final stage, the cell plate contacts and adheres to the plasma membrane of the parent cell. At the same time the tubular network expands to form a fenestrated sheet.

(E) At the end of mitosis, the phragmoplast disappears, the cell enters interphase, and microtubules reappear in the cytosol near the plasma membrane, where they play a role in the deposition of cellulose microfibrils during cell wall growth.



PLANT MIDDLE LAMELLA

The middle lamella is also called the cementing layer between two plant cells. It is composed of pectin layer. It cements the primary cell walls of two adjoining plant cells together.

Structure

In plants, the pectins form a unified and continuous layer between adjacent cells. Frequently, it is difficult to distinguish the middle lamella from the primary wall, especially in cells that develop thick secondary walls. In such cases, the two adjacent primary walls and the middle lamella, and perhaps the first layer of the secondary wall of each cell, may be called a compound middle lamella.

Formation

Middle lamella is the first formed layer which is deposited at the time of cytokinesis. The cell plate that is formed during cell division itself develops into middle lamella. The middle lamella is made up of calcium and magnesium pectates.

In most plants, homogalactouronans are the main components of middle lamella pectins. In grasses and lycopodiophytes, Rhanmogalactouronans are the main pectins in middle lamella.

Functional role of middle lamella

Plants need middle lamella to give adjacent cells stability and so that they can form plasmodesmata between the cells.

Plant cells stay together in a tissue tightly adhered to each other due to middle lamella. This type of association provides a specific type of structure and stability to the plant tissue.

It is an important determinant of fruit ripening. When a fruit transforms from its unripe state to ripe state, there is a considerable breakdown of middle lamella in the mesocarp part of the fruit. This makes fruits softer and more edible by the potential seed dispersal agent of the plant species.

FUNCTIONS OF CELL WALL

1. The chief function of cell wall is to provide mechanical strength to the plant cells. Like the exoskeleton of animals, cell wall acts like a skeletal framework of plants. Particularly in vascular plants, the cell walls provide the main supporting framework.

2. Despite its strength, the plant cell wall is fully permeable to water and solutes. This is because the matrix is riddled with minute water-filled channels through which free diffusion of water and water soluble substances such as gases, salts, sugars, hormones and like can take place. Moreover, the molecules of the matrix are strongly hydrophilic ("water-loving") with the result that in normal circumstances the cell wall is saturated with water like a sponge *(e.g.,* primary cell wall is ~70% percent water by weight).

3. The cross-linked structure of the cell wall is, however, found to slightly impede the diffusion of small molecules such as water, sucrose and K^+. The average diameter of the spaces between the cross linked macromolecule in most cell wall is about 5 nm, this is small enough to make the movement of any globular macromolecules with a M.W. much above 20,OOO daltons extremely slow. Therefore, plants subsist on molecules of low molecular weight, and any intercellular signaling molecules that have to pass through the cell wall must also be small and, water soluble: In fact, most of the known plant signaling molecules, such as growth regulating substances-auxins, cytokinins and gibberellinshave molecular weights of less than 500 daltons.

4. Lignification makes the cell wall much more rigid, and renders it impermeable. Once lignification is complete the protoplasm can no longer absorb materials from outside the cell, which, therefore, dies. Hence, *lignified tissue is always dead.* Thus, a lignified tissue becomes well adapted for two types of functions: (1) it provides the mechanical strength due to its ligno-cellulose composition. (2) it transports water and salts, since, lignification involves loss of the protoplasm resulting in the formation of a hollow waterproof tube of the xylem vessels or tracheids.

PLASMA MEMBRANE

What is a Plasma Membrane?

A Plasma Membrane is an ultra thin, elastic, living, dynamic fluid-mosaic, predominantly non-covalent supra-molecular assembly of amphipathic lipids, proteins and trace amounts of conjugated carbohydrates enclosing every type of cell, both prokaryotic and eukaryotic. It physically separates the cytoplasm from the surrounding extra-cellular environment and acts as a selective transport-barrier plus mediates a number of biologically important processes like signal transduction, enzymatic reactions, energy conversion, excitability etc.

The salient features of the Plasma Membrane include the following:

1. Sheet like structure which is only two molecules thick
2. Form closed boundaries around the cell. A similar boundary can be found outside certain viruses (enveloped viruses) too.
3. The thickness is about 100 Å (10 nm).
4. Consist mainly of lipids and proteins in approximately equal ratio. Plasma Membranes also contain carbohydrates that are linked to lipids and proteins. Carbohydrate is never found free in the membrane.
5. Predominantly Noncovalent assemblies. The constituent protein and lipid molecules are held together by many noncovalent interactions, which are cooperative. However, certain proteins are covalently attached to the membrane lipids (like Prenyl Anchored Proteins) and the Carbohydrates of the membrane are always covalently attached to a membrane lipid or a membrane protein.
6. Lipids are always amphipathic, that is they have both hydrophilic and hydrophobic parts. These lipids spontaneously form closed bimolecular sheets in aqueous media.
7. The structure of the Plasma Membrane is essentially formed by the amphipathic lipids whereas specific proteins mediate distinctive functions of Plasma Membranes. Proteins serve as pumps, channels, receptors, energy transducers, and enzymes.
8. Asymmetric structure. The two faces of Plasma Membranes always differ from each other.
9. Fluid structures. Lipid and protein molecules can diffuse from one location to another in the membrane.
10. Electrically polarized nature, such that the inside is negative [typically - 60 millivolts (mV)]. Membrane potential plays a key role in transport, energy conversion, and excitability.

Chemical Composition

Chemically, Plasma Membrane and other membranes of different organelles contain:

1. **Amphipathic lipids**, which are such lipids which contain both polar and non polar parts within the same molecule;
2. **Proteins**, which are almost always globular;
3. **Conjugated carbohydrates**, which are always covalently attached either to a membrane lipid or to a membrane protein.

The relative proportion of proteins and lipids in any membrane depends on the physiological role played by the membrane. The general underlying principle is that proteins will predominate in the membranes, which play an active metabolic role, such as chloroplast lamella or mitochondrial inner membrane. On the other hand, those membranes that primarily have an enveloping role will have greater proportion of lipids. As the Plasma Membranes serve both in compartmentalization and in biochemical reactions, they have nearly equal ratio of lipids and proteins. Carbohydrates are found in the Plasma Membrane only in trace amounts, and they may even be absent in many other membranes.

The following table illustrates this point.

MEMBRANE	LIPID %	PROTEIN %	CARBOHYDRATE %
Mouse liver Plasma Membrane	52	44	4
Amoeba Plasma Membrane	42	54	4
Human RBC Plasma Membrane	40	52	8
Chloroplast Lamella (mainly involved in bioenergetic conversion)	30	70	0
Mitochondrial Inner Membrane (mainly involved in bioenergetic conversion)	24	76	0
Myelin Sheath (mainly insulating function)	79	18	3

Plasma Membrane Lipids

Lipid—that is, fatty—molecules constitute about 50% of the mass of most animal cell membranes, nearly all of the remainder being protein. There are approximately 5×10^6 lipid molecules in a 1 μm × 1 μm area of lipid bilayer, or about 10^9 lipid molecules in the plasma membrane of a small animal cell. All of the lipid molecules in cell membranes are amphipathic (or amphiphilic)—that is, they have a hydrophilic ("water-loving") or *polar* end and a hydrophobic ("water-fearing") or *nonpolar* end.

Three major classes of **amphipathic lipids** [possessing both hydrophilic and hydrophobic domains] are commonly present in the Plasma Membrane and other bio-membranes:

1. Glycero Phospholipids (most abundant)
2. Sphingolipids
3. Sterols

The relative proportions of these lipids vary in different membranes.

Glycero-Phospholipids

The glycerophospholipids are made up of three components:

1. a phosphorylated head group,
2. a three-carbon glycerol backbone

3. two hydrocarbon fatty acid chains.

The phosphorylated head group is attached to *carbon-3 of the glycerol backbone*, while the two fatty acid chains are attached to the other two carbon atoms. The phosphorylated head group is a strongly polar group, while the fatty acid chain is a strongly non-polar group. This kind of structure makes GlyceroPhospholipids **amphipathic molecules**. *Due to this property, the Glycerophospholipids spontaneously form a bilayer in aqueous medium and thus give rise to the basic structure of the membrane.*

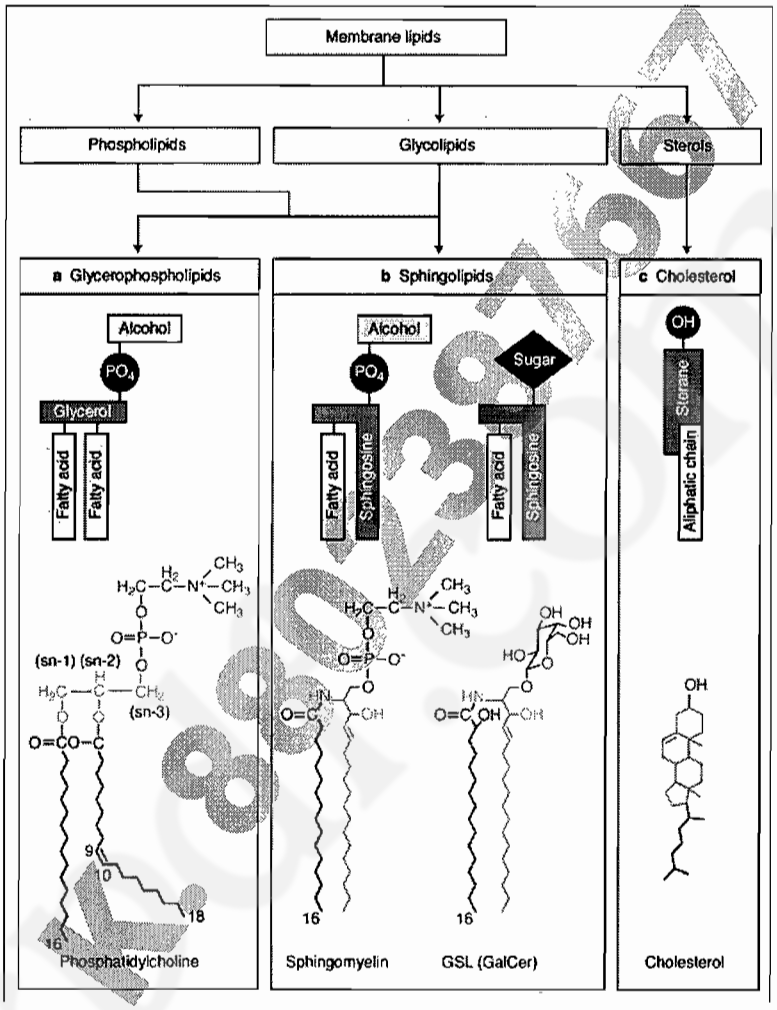


FIGURE 1: The three classes of membrane lipids

Usually the fatty acid chains in glycerophospholipids have an even number of carbon atoms (e.g. palmitate, C16; stearate, C18) and are unbranched. The chains can either be fully saturated with hydrogen atoms or be unsaturated and have one or more double bonds (e.g. oleate C 18:1 which has 18 carbon atoms and one double bond; arachidonic acid C 20:4 which has 20 carbon atoms and four double bonds). The presence of double bonds increase the fluidity of the membrane, because each double bond introduces a 45° bend in the hydrocarbon chain. Such bends interfere with compact packaging of the lipids. The major glycerophospholipids found in membranes include **phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine**, **phosphatidylglycerol**, and **phosphatidylserine**. **Diphosphatidylglycerol** (or **cardiolipin**) is found predominantly in the inner mitochondrial membrane.

Sphingolipids

Sphingolipids have a sphingosine backbone in place of the glycerol in glycerophospholipids. Like the glycerophospholipids, they also have a phosphorylated headgroup (either choline or ethanolamine) and two hydrocarbon chains. One of the hydrocarbon chains comes from the sphingosine molecule, the other is a fatty acid as found in the glycerophospholipids. **Sphingomyelins** are the commonest Sphingolipids. They are particularly abundant in the myelin sheath that surrounds nerve cells.

The glycosphingolipids, such as the **cerebrosides** and **gangliosides**, are also derived from sphingosine, but in place of the phosphorylated. headgroup they have one or more sugar residues. The **galactocerebrosides**

have a single galactose residue and are found predominantly in the neuronal cell membranes of the brain.

The sphingolipids, as it has been recently discovered, are closely associated with sterols in the plasma membrane and make a dense and floating lipid aggregate, called ***membrane rafts***.

Sterols

The sterol **cholesterol** is a major constituent of animal Plasma Membranes but is absent from prokaryotes. Plants contain little cholesterol but have instead a number of other sterols, mainly **Stigmasterol, Camposterol, Cycloartenol, Coniferol,** and **Sitosterol.** The bacterial cells do not have true sterols but they have structural analogs, called **Hopanoids**.

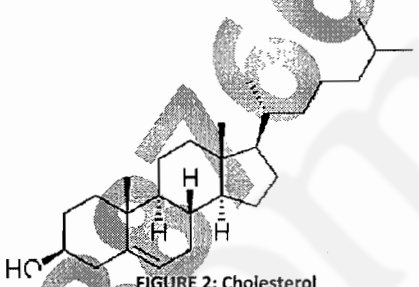


FIGURE 2: Cholesterol

The fused ring system of sterols means that they are more rigid than other membrane lipids. By this property, they play an important role in stabilizing the membrane fluidity.

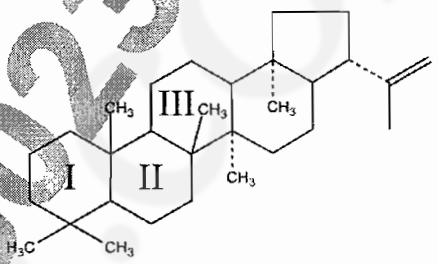


FIGURE 3: A Hopanoid Compound

Plasma Membrane Proteins

The amount and types of proteins in the membranes are highly variable. *Almost all the proteins of the Plasma Membrane are Globular.*

The proteins of the Plasma Membrane are not classified according to their biochemical properties, as the membrane lipids are classified. The classification of the membrane proteins is done on the basis of their relative position and association with respect to the membrane lipids.

In the Fluid Mosaic Model proposed by Singer & Nicholson in 1972, the proteins were classified into two main types according to their position in the Plasma Membrane: **integral** or **intrinsic proteins** and **peripheral** or **extrinsic proteins**. The intrinsic proteins associate firmly with the inner parts of membrane lipid bilayer and they can only be separated only by harsh enzymatic & detergent treatment. On the other hand, the extrinsic proteins have a weaker association and are bound to lipids of membrane by electrostatic interaction.

However, based on the current understanding of the *various ways in which membrane proteins associate with the lipid bilayer*, 10 types of membrane proteins are now classified. In Figure 4, eight different types of membrane proteins are displayed. (Plesae refer to Class Lecture Notes, for a simpler depiction of the various types of membrane proteins).

The eight types of proteins designated numerically in Fig 4 are**:** (1) Single α helix protein (2) Multiple-pass α helical protein (3) β barrel protein (4) Monotopic protein (5) Prenyl anchored protein (6) GPI (Glycophosphatidylinositol) anchored protein on the exoplasmic side (7) Proteins attached proteins (cytoplasmic side) (8) Proteins attached proteins (exoplasmic side).

The Multisubunit proteins and Peripheral proteins attached on the surface of the polar lipid heads are not shown in this figure (please refer to your Class Lecture illustrations).

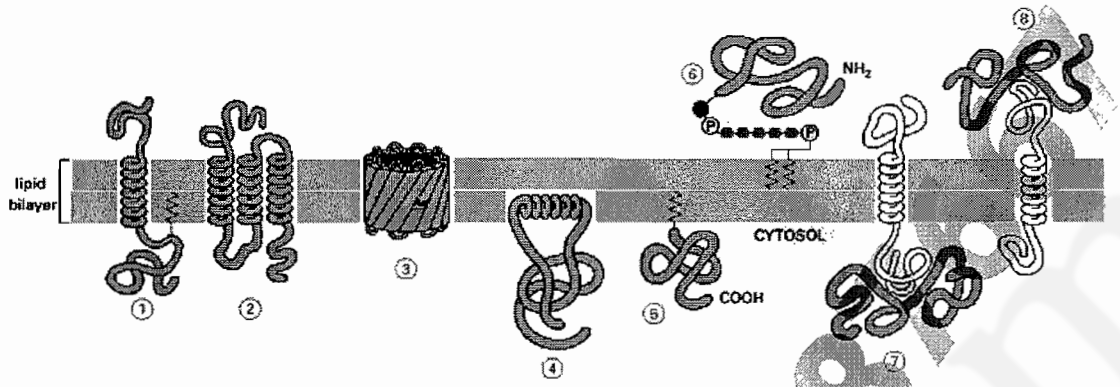


FIGURE 4: The principal types of plasma membrane proteins.

There is one more way of classifying the membrane proteins, which is on the functional basis. Based on their functions, proteins of Plasma Membrane can also be classified into eight main types:

1. Structural proteins for example Cytoskeleton anchoring proteins, ECM attachment proteins etc.
2. Enzymes like Adenylyl Cyclase
3. Transport proteins (permeases or carriers) like Na^+/K^+ Pump
4. Signal receptors like G-protein coupled receptors
5. Signaling proteins like Notch proteins
6. Coat proteins like Clathrin and Caveolin
7. Embedded antibodies
8. Cell-to cell attachment proteins

Membrane Carbohydrates

Carbohydrates are present only in the Plasma Membrane and in no other organellar membrane except the Endoplasmic Reticulum membrane. They are present as short, unbranched or branched chains of sugars (oligosaccharides) attached either to exterior ectoproteins (forming glycoproteins) or to the polar ends of phospholipids at the external surface of the Plasma Membrane (forming glycolipids). Almost no carbohydrate is located at the cytoplasmic or inner surface of the Plasma Membrane. All types of oligosaccharides of the Plasma Membrane are formed by various combinations of six principal sugars (all of which are glucose-derivatives) :D-galactose, D-mannose, L-fucose, N-acetylneuraminic acid (also called sialic acid), N-acetyl-D-glucosamine and N-acetyl-D-galactosamine.

Molecular Architecture of Plasma Membrane

On a molecular level PM is considered a two dimensional structure by biochemists because of its extremely thin [~ 10nm] structure. The molecular structure of PM has been a field of intense research and a number of models have been proposed from time to time to explain it (from **Langmuir in 1917** to **Unwin & Henderson in 1975**).

The FLUID MOSAIC MODEL proposed by Singer & Nicholson in 1972, with modifications by Unwin & Henderson in 1975 is now the universally accepted model for the molecular architecture of not only the Plasma Membrane but for all the biomembranes.

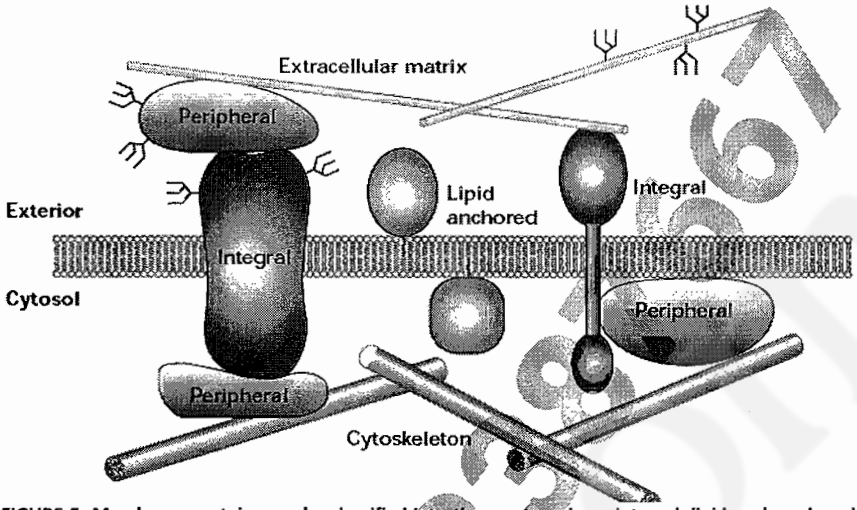


FIGURE 5: Membrane proteins can be classified into three categories— integral, lipid-anchored, and peripheral—on the basis of the nature of the membrane–protein interactions

The fluid mosaic model has two key features, both implied by its name. Simply put, the model envisions a membrane as a structure in which a mosaic of proteins is discontinuously embedded in or attached to a fluid lipid bilayer. Such a situation is shown in the figure here.

The Singer-Nicholson model retained the basic lipid bilayer structure of earlier models of plasma membranes. But they viewed membrane proteins in an entirely different way that is as discrete globular entities that associate with the membrane on the basis of their affinity for the hydrophobic interior of the lipid bilayer. The model turned out to be in conformity with the experimental data & Electron Microscope images. The most direct experimental validation of the Fluid Mosaic Model comes from Electron Microscopy of a Freeze Fractured Membrane.

The lipid bilayer is thermodynamically the most stable structure, when amphipathic lipids are subjected to an aqueous medium. A bilayer exposes the polar hydrophilic parts to the surrounding aqueous medium while protecting the Non polar tail from water. Among the three groups of membrane lipids, the glycerophospholipids, being cylindrical, most spontaneously form bilayers in aqueous environments. *It is this bilayer that forms the structural basis of membranes.*

The fluidity of the membrane, a primary feature of the Singer-Nicholson model, means that most of the lipid components of a membrane are in constant motion, capable of lateral mobility (i.e., movement parallel to the membrane surface). Lipid molecules readily exchange places with their neighbours within a monolayer (~107 times a second).

Because of fluidity, many membrane proteins are also able to move laterally within the membrane. Fluidity of the membrane can be demonstrated by cell fusion experiments.

Apart from lateral movement, the membrane lipids are also known to show four other types of movements.

1. Bobbing
2. Rotation
3. Flexion
4. Flipping

Based on differences in the nature of protein linkage to the lipid bilayer, the Fluid Mosaic Model identifies

two broad classes of membrane proteins.

1. **Integral membrane** proteins, also called transmembrane proteins, span a phospholipid bilayer and are built of three segments. The cytosolic and exoplasmic domains have hydrophilic exterior surfaces that interact with the aqueous solutions on the cytosolic and exoplasmic faces of the membrane. These domains resemble other water-soluble proteins in their amino acid composition and structure. In contrast, the 3-nm-thick membrane-spanning domain contains many hydrophobic amino acids whose side chains protrude outward and interact with the hydrocarbon core of the phospholipid bilayer. In all transmembrane proteins examined to date, the membrane-spanning domains consist of one or more helices or of multiple alpha-strands. In addition, most trans-membrane proteins are glycosylated with a complex branched sugar group attached to one or several amino acid side chains. Invariably these sugar chains are localized to the exoplasmic domains.
2. **Peripheral proteins**, which are much more hydrophilic and are therefore located on the surface of the membrane, where they are linked non-covalently to the polar head groups of phospholipids. They are also bound to the membrane indirectly by interactions with integral membrane proteins. Peripheral proteins are localized to either the cytosolic or the exoplasmic face of the plasma membrane.

Soon after the enunciation of the Fluid Mosaic Model, it was realized that membrane proteins could be classified into three categories— integral, lipid-anchored, and peripheral—on the basis of the nature of the membrane–protein interactions (Figure 5).

Lipid-anchored membrane proteins are bound covalently to one or more lipid molecules. The hydrophobic carbon chain of the attached lipid is embedded in one leaflet of the membrane and anchors the protein to the membrane. The polypeptide chain itself does not enter the phospholipid bilayer.

Regulation of Membrane Fluidity

Role of unsaturated fats in increasing membrane fluidity: Double bonds in unsaturated hydrocarbon chains tend to increase the fluidity of a phospholipid bilayer by making it more difficult to pack the chains together in an ordered crystalline structure. The presence of double bonds increases the fluidity of the membrane, because each double bond introduces a 45° bend in the hydrocarbon chain. Such bends interfere with compact packaging of the lipids. Thus, to maintain fluidity of the membrane, cells of organisms living at low temperatures have high proportions of unsaturated fatty acids in their membranes, than do cells at higher temperatures.

In fact, certain membrane transport processes and enzyme activities cease when the lipid bilayer's viscosity increases beyond a threshold level. In contrast, if lipid bilayer's fluidity is increased, the membrane's receptors for the hormone are withdrawn from the cell surface, thereby hampering hormone action.

***Role of cholesterol in maintaining fluidity of membrane*:** Eukaryotic Plasma Membranes contain a large amount of cholesterol. Cholesterol molecules orient themselves in the lipid bilayer in such a way that their hydroxyl groups remain close to polar head groups of the phospholipids, their rigid plate-like steroid rings interact with and partly immobilise those regions of hydrocarbon chains that are closest to the polar head groups, leaving the rest of the chain flexible. Cholesterol inhibits phase transition by preventing hydrocarbon chains from coming together and crystallizing. Cholesterol also tends to decrease the permeability of lipid bilayers to small water-soluble molecules and is thought to enhance both the

flexibility and the mechanical stability of the bilayer.

Cold acclimation in plants leads to an increase in the ratio of phospholipids to sterols in the Plasma Membrane of most plants. In extremely cold-hardy woody species is cold acclimation correlated with a strong increase in unsaturated fatty acids, particularly linoleic acid (18:2). These changes in lipid composition result in a higher fluidity of the lipid bilayer, and thus a lower freezing point, enabling the Plasma Membrane to function properly at low temperature.

***Constraints on the Mobility of Membrane Molecules*:** In the fluid mosaic Plasma Membrane, there is not complete and independent freedom of movement for its different component molecules. The mobility of some part of lipid molecules is constrained since that remains tightly bound to some of the integral membrane proteins. For example, the mobility of lipid molecules surrounding cytochrome oxidase are immobilized by the enzyme and makes boundary lipid layer.

In contrast to lipids, the mobility and distribution of protein molecules in the membrane is controlled by various ways: (1) Certain proteins of membrane are constrained by protein-protein interactions to form specialized ordered regions (2) Certain peripheral proteins (endoproteins) may form a bridge-like lattice work between integral proteins and restrict their lateral mobility ,e.g., spectrin-ankyrin-actin cytoskeletal meshwork provides a rigidity to the membrane of human erythrocytes and does not permit the clustering or capping of integral proteins when the appropriate antibodies or lee tins are added. (3) In nucleated eukaryotic cells, the mobility of the peripheral endoproteins and integral proteins is restrained by their attachment to the ectoplasmic cytoskeleton. The cytoskeleton is extensive, including myosin filaments, actin filaments and microtubules.

The Functions of Plasma Membrane

The plasma membrane has many common functions in all cells. Although the lipid composition of a membrane largely determines its physical characteristics, its complement of proteins is primarily responsible for a membrane's functional properties.

1. In all cells, the plasma membrane acts as a permeability barrier that prevents the entry of unwanted materials from the extracellular milieu and the exit of needed metabolites. Specific membrane transport proteins in the plasma membrane permit the passage of nutrients into the cell and metabolic wastes out of it; others function to maintain the proper ionic composition and pH (≈7.2) of the cytosol. The structure and function of transport proteins make the plasma membrane selectively permeable to different molecules. The plasma membrane is highly permeable to water but poorly permeable to salts and small molecules such as sugars and amino acids. Owing to osmosis, water moves across such a semipermeable membrane from a solution of low solute (high water) concentration to one of high solute (low water) concentration until the total solute concentrations and thus the water concentrations on both sides are equal.
2. The plasma membrane is intimately engaged in the assembly of cell walls, which in plants are built primarily of cellulose. The cell wall prevents the swelling or shrinking of a cell that would otherwise occur when it is placed in a hypotonic or hypertonic medium.
3. In animal cells, specialized areas of the plasma membrane contain proteins and glycolipids that form specific junctions between cells to strengthen tissues and to allow the exchange of metabolites between cells. Certain plasma-membrane proteins anchor cells to components of the extracellular matrix, the mixture of fibrous proteins and polysaccharides that provides a bedding on which most sheets of epithelial cells or small glands lie.

4. Many proteins in the plasma membrane act as anchoring points for many of the cytoskeletal fibers that permeate the cytosol, imparting shape and strength to cells.
5. The plasma membranes of many types of eukaryotic cells also contain receptor proteins that bind specific signaling molecules (e.g., hormones, growth factors, neurotransmitters), leading to various cellular responses. These proteins, which are critical for cell development and functioning.
6. Peripheral cytosolic-side proteins that are recruited to the membrane surface function as enzymes, intracellular signal transducers, and structural proteins for stabilizing the membrane.

Like the plasma membrane, the membrane surrounding each organelle in eukaryotic cells contains a unique set of proteins essential for its proper functioning.

CELL ADHESION

Intercellular recognition and cell adhesion

The ability of cells to recognize and adhere to one another plays an important role in cell survival and reproduction. For example, when starved, several types of single-cell organisms band together to develop the specialized cells needed for reproduction. In this process, certain cells at the centre of the developing aggregate secrete chemicals that cause the other cells to adhere tightly into a group. In the case of slime mold amoebas, starvation causes the secretion of a compound, cyclic adenosine monophosphate (cyclic AMP, or CAMP), that induces the cells to stick together end to end. With further aggregation, the cells produce another cell-surface glycoprotein with which they stick to one another over their entire surfaces. The cellular aggregates then produce an extracellular matrix, which holds the cells together in a specific structural form.

Tissue and species recognition

Some multicellular animals or tissues can be dissociated into suspensions of single cells that show the same cellular recognition and adhesion as do aggregates of single-cell organisms. The marine sponge, for example, can be sieved through a mesh, yielding single cells and cells in clumps. When this cell suspension is rotated in culture, the cells reaggregate and in time reform a normal sponge. This reassociation shows selective cell recognition; that is, only cells of the same species reassociate. The ability of the cells to distinguish cells of their own species from those of others is mediated by proteoglycan molecules in the extracellular matrix. The proteoglycan binds to specific cell-surface receptor sites that are unique to a single species of sponge.

Cells from tissues of vertebrate animals can, like sponge cells, be dissociated and allowed to reaggregate. For example, when vertebrate embryonic cells from two different tissues are dissociated and then rotated together in culture, the cells form a multicellular aggregate within which they sort according to the type of tissue, a sorting that occurs regardless of whether the cells are from the same or different species. The specificity is due to a set of cell-surface glycoproteins called cell adhesion molecules (CAM). A portion of the CAM that extends from the surface of a cell adheres to identical molecules on the surface of adjacent cells. These CAM appear early in embryonic life, and their amounts in tissues change as the organs develop. The CAM, however, are not responsible for the stable adhesion of one cell to another; this more permanent adhesion is carried out by cell junctions.

Cell junctions

There are three functional categories of cell junction: adhering junctions, often called desmosomes; tight, or occluding, junctions; and gap, or permeable, junctions. Adhering junctions hold cells together mechanically and are associated with intracellular fibres of the cytoskeleton. Tight junctions also hold cells together, but they form a nearly leakproof intercellular seal by fusion of adjacent cell membranes. Both adhering junctions and tight junctions are present primarily in epithelial cells. Many cell types also possess gap junctions, which allow small molecules to pass from one cell to the next through a channel.

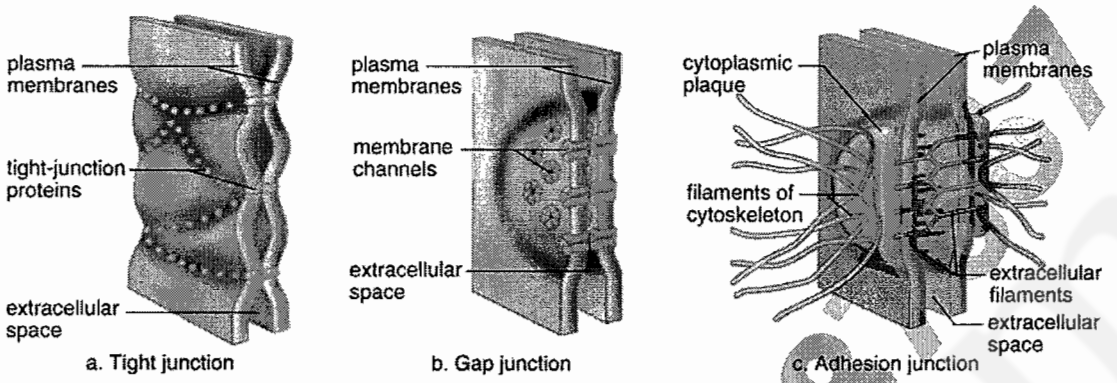


FIGURE 1: TYPES OF CELL JUNCTIONS

Adhering junctions

Cells subject to abrasion or other mechanical stress, such as those of the surface epithelia of the skin, have junctions that adhere cells to one another and to the extracellular matrix. These adhering junctions are called desmosomes when occurring between cells and hemidesmosomes (half-desmosomes) when linked to the matrix. Adhering junctions distribute mechanical shear force throughout the tissue and to the underlying matrix by virtue of their association with intermediate filaments crossing the interior of the cell. The linkage of these filaments, also called keratin filaments, to the desmosomes and, through these junctions, to adjacent cells provides a nearly continuous fibrous network throughout an epithelial sheet. Adhering junctions are also seen in other types of cells—for example, in the muscles of the heart and uterus—allowing these cells to remain anchored together despite the contractions of the muscles.

Tight junctions

Sheets of cells separate fluids within the organs from fluids outside, as in the epithelial layer lining the intestine. This separation requires leakproof junctions between cells. Tight junctions form leakproof seals by fusing the plasma membranes of adjacent cells, creating a continuous barrier through which molecules cannot pass. The membranes are fused by tight associations of two types of specialized integral membrane proteins, in turn repelling large water-soluble molecules. In invertebrates this function is provided by septate junctions, in which the proteins of the membrane rather than the lipids form the seal.

Gap junctions

These junctions allow communication between adjacent cells via the passage of small molecules directly from the cytoplasm of one cell to that of another. Molecules that can pass between cells coupled by gap junctions include inorganic salts, sugars, amino acids, nucleotides, and vitamins but not large molecules such as proteins or nucleic acids.

Gap junctions are crucial to the integration of certain cellular activities. For example, heart muscle cells generate electrical current by the movement of inorganic salts. If the cells are coupled, they will share this electrical current, allowing the synchronous contraction of all the cells in the tissue. This coupling function requires the regulation of molecular traffic through the gaps. The junctions are not open pores but dynamic channels, which change their permeability with changes in cellular activity. They consist of proteins completely crossing the cell membrane as six-sided columns with central pores. Under certain conditions the proteins are thought to change shape, causing the pores to become smaller or larger and thus changing the permeability of the junction.

Gap junctions are also found in tissues that are not electrically active. In these tissues, the junctions allow nutrients and waste products to travel throughout the tissue. Cells in such tissues are said to be metabolically coupled. During the formation of embryos, gap junctions are crucial to establishing differences between separate groups of cells, the coupled cells undergoing development together to become a specialized tissue.

MEMBRANE TRANSPORT

Introduction to membrane transport

The plasma membrane is a selectively permeable barrier between the cell and the extracellular environment. Its permeability properties ensure that essential molecules such as ions, glucose, amino acids, and lipids readily enter the cell, metabolic intermediates remain in the cell, and waste compounds leave the cell. In short, the selective permeability of the plasma membrane allows the cell to maintain a constant internal environment.

To summarize, the transport regulation across a membrane is crucially important because:

1. It allows the cell to maintain concentrations of solutes in its cytosol and in the intracellular membrane-enclosed compartments.
2. It allows optimal amounts of water to be present in the cell.
3. It is essential to ingest essential nutrients.
4. It enables the cell to excrete metabolic waste products.
5. It regulates intracellular ion concentrations.
6. In some cases, it enables the entry and exit of signaling molecules or hormones.

It is estimated that about 15 – 20% of the total ~25,000 human genes encode various transport proteins.

Types of transport across membranes

The best classification of transport across a membrane is done based on bioenergetics of the process. There are two principal ways of substrate transport across a membrane.

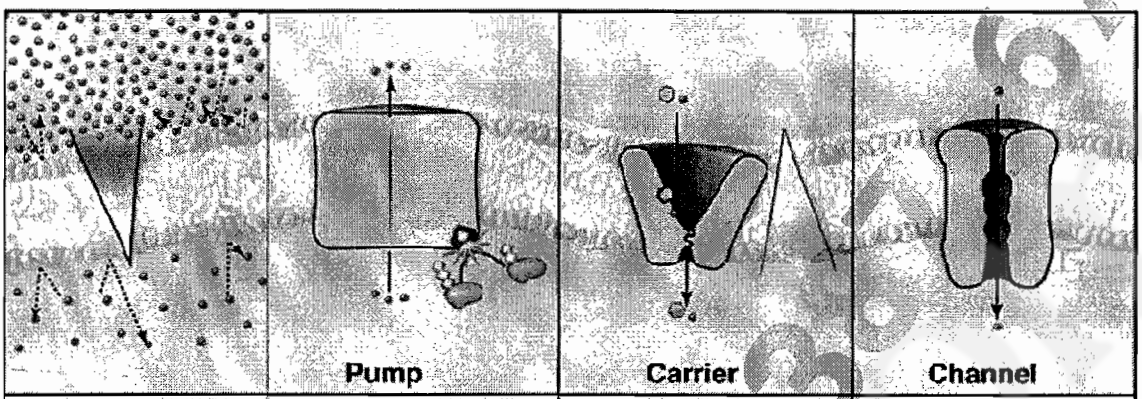
1. **Passive process**: No consumption of biological free energy; the process of transport is powered by electrochemical gradient present within the system itself. May occur with or without transport proteins.
2. **Active process**: Requires a supply of biological free energy, provided either by ATP hydrolysis or Proton Motive Force (PMF).

The mechanism behind various transport methods

General Principles

There are two principal ways of substrate transport across a membrane; Passive Transport and Active Transport (as described before).

Although lipid bilayers provide a barrier to diffusion of ions and polar molecules larger than about 150 D, protein pores provide selective passages for ions, and other larger molecules across membranes.



	Pump	Carrier	Channel
Specificity	Absolute	Intermediate	Only 10–20×
Rate (ions/s)	100	<1000	10^6
Gradient	Uphill	Downhill*	Downhill
Energy input	Required	No	No
Ions/conformational change	~1	~1	Many

*May pull another solute uphill

FIGURE 1: Properties of the three types of proteins that transport ions and other solutes across membranes. The triangle represents the concentration gradients across the membrane.

Integral proteins that control membrane permeability fall into *three broad classes*-pumps, carriers, and channels-each with distinct properties (Fig. 1). These proteins allow cells to control solute traffic across membranes, an essential feature of many physiological processes.

1. **Pumps** are enzymes that utilize energy from adenosine triphosphate (ATP), light, or (rarely) other sources to move ions (generally, cations) and other solutes across membranes at relatively modest rates. They establish concentration gradients between membrane-bound compartments.

2. **Carriers** or **Transporters** are enzyme-like proteins that provide passive pathways for solutes to move across membranes down their concentration gradients from a region of higher concentration to one of lower concentration. Each conformational change in a carrier protein translocates a limited number of solutes across the membrane. Carriers use ion gradients as a source of energy to perform a remarkable variety of work. Some carriers use translocation of an ion down its concentration gradient to drive another ion or solute up a concentration gradient.

3. **Channels** are ion-specific pores that typically open and close transiently in a regulated manner. When a channel is open, a flood of ions passes quickly across the membrane through the channel, driven by electrical and concentration gradients. The movement of ions through open channels controls the electrical potential across membranes, so that changes in channel activity produce rapid electrical signals in excitable membranes of nerves, muscles, and other cells.

Passive Transport

A molecule or ion that crosses the membrane by moving down a concentration or electrochemical gradient and without expenditure of metabolic energy is said to be transported passively. Another name for this

process is **diffusion**. All molecules and ions are in constant motion and it is the energy of motion - kinetic energy - that drives passive transport.

Passive transport is dependent on:

1. the permeability of the cell membrane
2. size of the substrate (smaller the size, the greater will be rate of passive transport)
3. concentration or electrochemical gradient (the higher the gradient is, the faster will be the transport)

The two main kind of passive transport are:

1. simple diffusion
2. facilitated diffusion

Simple Diffusion

This is the diffusion *without any protein involvement*. The substrate passes through the lipid bilayer. Diffusion is the net movement of material from an area of high concentration of that material to an area with lower concentration. The difference of concentration between the two areas is often termed as the *concentration gradient*, and diffusion will continue until this gradient has been eliminated. Strictly speaking, therefore, diffusion always proceeds from regions of higher to lower free energy.

Simple diffusion across a membrane depends on the following considerations.

1. The substrate must be of small size, because it has to pass through the lipid bilayer – making way between the fatty acid tails of membrane glycerophospholipids.
2. The substrate should preferably be non polar.
3. The substrate should preferably be uncharged.
4. The substrate should preferably be lipid soluble.
5. There must be a concentration gradient.
6. There should be larger general diffusion area.
7. The diffusion distance should not be too long.

Simple Diffusion is biologically important because it enables the exchange of respiratory gases, namely Oxygen and Carbon di-oxide. It also allows about 20% of total water flux through a biological membrane.

The advantages of simple diffusion are:

1. Energetically inexpensive, because no biological energy is consumed
2. The entire general membrane surface acts as a diffusion surface.
3. The process is relatively simple.

4. Since it does not depend on any protein, it shows no saturation effect or sensitivity towards toxins.

The limitations of simple diffusion are that it:

1. is a slow process
2. is a non specific process
3. cannot transport polar, charged or large substrates.
4. always depends on the concentration gradient

Facilitated Diffusion

Facilitated diffusion, (facilitated transport) is a process of diffusion in which molecules diffuse across membranes *with the assistance of transport proteins*. Facilitated diffusion is essentially a passive process does not require biological free energy and carries molecules or ions down a concentration gradient.

The need for facilitated diffusion: Small uncharged molecules can easily diffuse across cell membranes. However, due to the hydrophobic nature of the lipids, water-soluble molecules and ions cannot do so; instead, they are helped across by transport proteins.

The process of facilitated diffusion: *Four major groups of integral membrane proteins* are involved in facilitated diffusion.

1. **Carriers or Transporters,** which bind a specific type of solute and are thereby induced to undergo a series of conformational changes which has the effect of carrying the solute to the other side of the membrane. The transport protein involved is intrinsic, completely spanning the membrane. It has a binding site for the specific substrate that has to be transported. After binding to the molecule, the protein changes shape and carries the molecule across the membrane, where it is released. The protein then returns to its original shape, to wait for more molecules to transport.

This mechanism of functioning is called Conformational Change Mechanism (given by Prof. Jonathan Singer and co-workers in 1970s). The best known example of facilitated diffusion is Glucose transport in animal cells (Figure 2) through the glucose transpoters (GLUTs) for which atleast five isoforms are known in humans – from GLUT1 – GLUT 5.

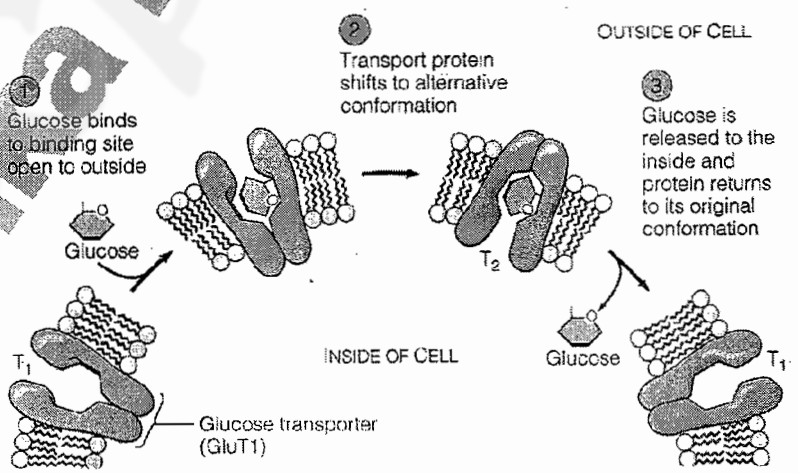


FIGURE 2: The working of GLUT

Another example is Band 3, the anion transporter, which facilitates transport of bicarbonate and chloride ions. The transport proteins participating in facilitated diffusion resemble enzymes. Just as enzymes are substrate specific and only catalyze certain substrates, transport proteins are solute specific and only transport certain solutes. Transport proteins also have a limit of how many solutes they can transport that they cannot exceed. This is called the saturation effect. Finally, certain molecules can inhibit the protein in a way similar to inhibition in enzymes.

The transporters can further be classified into:

A. Uniporters when at a time they allow only one type of substrate

B. Co-transporters when two solutes are transported simultaneously and their transport is coupled such that transport of either stops if the other is absent.

In cotransport, the process is called symport if the two solutes are moved in the same direction or antiport if the two solutes are moved in opposite directions. In the erythrocyte plasma membrane the example of the uniport transporter is GLUT1 and the example of antiport transporter is the anion transporter Band3 protein.

2. **Ion Channels** do not really bind the solute, but they have hydrophilic pores through which certain types of solutes can pass through. The channels are quite specific for the type of solute they will transport.

Most of the ion channels allow passage of only one kind of ion, so separate channels are needed for transporting such ions as Na^+, K^+, Ca^{2+}, and Cl^-. Such selectivity is remarkable and it may involve both *ion-specific binding sites* and a *constricted center that serves as a size filter*. The rate of transport is often quite high, a single channel can conduct almost a million ions per second.

Most ion channels have gates, which means that they can be opened and closed by conformational changes in the protein, thereby regulating the flow of ions through the channel.

In animal cells, three different kinds of stimuli induce conformational changes in gated channels:

A. Voltage gated channels open and close in response to change in the membrane potential; for example: As an impulse passes down a neuron, the reduction in the voltage opens sodium channels in the adjacent portion of the membrane. This allows the influx of Na^+ into the neuron and thus the continuation of the nerve impulse.

B. Ligand-gated channels are triggered by the binding of specific substances to the channel protein; such as Acetylcholine, GABA, cAMP, and cGMP.

C. Mechanosensitive channels respond to mechanical forces that act on the membrane. (For example: Sound waves bending the cilia-like projections on the hair cells of the inner ear open up ion channels leading to the creation of nerve impulses that the brain interprets as sound.)

Regulation of ion movement across membranes plays an important role in many types of cellular communication. For example, ion channels allow currents to be carried across the membrane and are thus of particular importance in the physiology of excitable cells like neurons and muscle cells. The transmission of electrical signals by nerve cells depends critically on rapid, controlled

changes in the movement of Na^+ and K^+ ions through their respective channels. In addition to such short-term regulation, most ion channels are also subject to longer-term regulation, usually in response to external stimuli such as hormones.

A growing number of human diseases have been discovered to be caused by inherited mutations in genes encoding channels.

Some examples:

Chloride-channel diseases

- Cystic fibrosis
- inherited tendency to kidney stones (caused by a different kind of chloride channel than the one involved in cystic fibrosis)

Potassium-channel diseases

- some inherited life-threatening defects in the heartbeat
- a rare, inherited tendency to epileptic seizures in the newborn.
- several types of inherited deafness

Sodium-channel diseases

- inherited tendency to certain types of muscle spasms
- Liddle's syndrome. Inadequate sodium transport out of the kidneys, because of a mutant sodium channel, leads to elevated osmotic pressure of the blood and resulting hypertension (high blood pressure).

3. **Pores:** Pores are formed by transmembrane proteins called porins and allow selected hydrophilic solutes with molecular weights up to about 600 Da to diffuse across the membrane. Compared with ion channels, the pores found in the outer membranes of mitochondria, chloroplasts, and many bacteria are somewhat larger and much less specific. These pores are formed by multiphase rnasmembrane proteins called porins. Bacterial porins are among the few trans-membrane proteins whose structures have been determined by X-ray crystallography. A key feature revealed by this technique is that the trnasmembrane segments of poring molecules cross the membrane not as an α helix but as a closed cylindrical β sheet called a β- barrel. The β-barrel has a water-filled pore at its center. The pore allows passage of various hydrophilic solutes, with the size limit for the solute molecules determined by the pore size of the particular porin.

4. **Aquaporins:** Aquaporins (AQPs) or the "*water pores*" are membrane proteins that function as water-specific channels. The first aquaporin, 'aquaporin-1', was reported by Peter Agre, from Johns Hopkins University in 1992. Agre and his colleagues received Nobel Prize in Chemistry in 2003. Aquaporins form tetramers (with each monomer having 6 membrane spanning alpha-helical domains) in the cell membrane, and facilitate the transport of water and, in some cases, other small solutes, such as glycerol, across the membrane. However, the water pores are completely impermeable to charged species, such as protons.

Aquaporins in mammals: More than 10 mammalian aquaporins have so far been identified, but the existence of many more is suspected. Most aquaporins appear to be exclusive water channels that will not allow permeation of ions or other small molecules. Some aquaporins - known as aquaglyceroporins - transport water plus glycerol and a few other small molecules. Aquaporins play a key role in control of water excretion by the kidney. Vasopressin (also known as "antidiuretic hormone") is a circulating peptide that regulates aquaporin-2 to result in variable and tightly regulated water excretion. When vasopressin levels rise in the blood, the collecting duct cells in the kidneys bind more vasopressin, initiating a complex signaling process, which results in movement of aquaporin-2-containing intracellular vesicles to the plasma membrane. These vesicles fuse with the plasma membrane, thus increasing the water permeability of the cells and allowing increased return of water from the nascent urine to the blood.

Aquaporins are also found in plants, predominantly in the Plasma Membrane and the Vacuolar Membrane.

Active Transport

Active transport uses specific transport proteins, called **pumps**. Protein pumps transport ions and other solutes across membranes up concentration gradients as great as 1 million-fold. Energy for this task can come from a variety of sources: light, oxidation-reduction reactions, or, most commonly, hydrolysis of ATP.

Pumps are also called **primary active transporters** because they transduce electromagnetic or chemical energy directly into transmembrane concentration gradients.

Osmosis is the diffusion of a liquid (most often assumed to be water, but it can be any liquid solvent) through a semi-permeable membrane from a region of high water potential to a region of low water potential. The semi-permeable membrane is one that is permeable to the solvent, but not to the solute, resulting in a water potential gradient across the membrane.

Although water is a polar molecule, it is able to pass through the lipid bilayer of the plasma membrane. Aquaporins form the hydrophilic channels accelerate the process, but even without these, water is still able to get through. Water passes by diffusion from a region of higher to a region of lower water-potential. Water is never transported actively; that is, it never moves against its concentration gradient. However, the concentration of water can be altered by the active transport of solutes and in this way the movement of water in and out of the cell can be controlled. (Example: The reabsorption of water from the kidney tubules back into the blood depends on the water following behind the active transport of Na^+).

Osmotic fluxes of water are governed by tonicity relations which are of three types.

1. If the potential of water in the medium surrounding a cell is greater than that of the cytosol, the medium is said to be **hypotonic**. Water enters the cell by osmosis. A red blood cell placed in a hypotonic solution (e.g., pure water) bursts immediately ("hemolysis") from the influx of water. Plant cells and bacterial cells avoid bursting in hypotonic surroundings by their strong cell walls. These allow the buildup of **turgor** within the cell. When the turgor pressure equals the osmotic pressure, osmosis ceases.
2. When red blood cells are placed in a 0.9% salt solution, they neither gain nor lose water by osmosis. Such a solution is said to be **isotonic**. The extracellular fluid (ECF) of mammalian cells is isotonic to their cytoplasm. This balance must be actively maintained because of the large number of organic molecules dissolved in the cytosol but not present in the ECF. These organic molecules exert an osmotic effect that, if not compensated for, would cause the cell to take in so much water that it would swell and might even burst. This fate is avoided by pumping sodium ions out of the cell with the Na^+/K^+ ATPase.
3. If red cells are placed in sea water (about 3% salt), they lose water by osmosis and the cells shrivel up. Sea water is **hypertonic** to their cytosol. Similarly, if a plant tissue is placed in sea water, the cell contents shrink away from the rigid cell wall. This is called **plasmolysis**. Sea water is also hypertonic to the ECF of most marine vertebrates. To avoid fatal dehydration, these animals (e.g., bony fishes like the cod) must continuously drink sea water and then desalt it by pumping ions out of their gills by active transport. (Marine reptiles — turtles and snakes — use special salt glands for the same purpose.)

Table 1 below summarizes the diversity of membrane pumps.

Table1. DIVERSITY OF MEMBRANE PUMPS

Energy Source	Pump	Driven Substance	Distribution
Light	Bacteriorhodopsin	H^+	Halobacteria
	Halorhodopsin	Cl^-	Halobacteria
Light	Photoredox	H^+	Photosynthetic organisms
Redox potential	Electron transport chain NADH oxidase	H^+	Mitochondria, bacteria
		Na^+	Alkalophilic bacteria
Decarboxylation	Ion-transporting decarboxylases	Na^+	Bacteria
Pyrophosphate	H^+-pyrophosphatase	H^+	Plant vacuoles, fungi, bacteria
ATP	Transport ATPases	Various ions and solutes	Universal

A very common example is found in both vertebrates and invertebrates, where the concentration of sodium ion is about 10 to 20 times higher in the blood than within the cell. The concentration of the potassium ion is the reverse, generally 20 to 40 times higher inside the cell. The **sodium-potassium pump** maintains such differential concentration inside the cell.

It is one of the best-studied examples of pump proteins. It is a cation exchange pump driven by energy of one ATP molecule to export three Na^+ ions outside the cell in exchange of the import of two K^+ ions inside the cell. It is a transmembrane protein which is a dimer having two subunits: one smaller unit which is a glycoprotein of 50,000 daltons M.W., having an unknown function; and another larger unit having 1,20,000 daltons M. W. The larger subunit of Na+- K+- ATPase performs the actual function of cation transport. Electrical organs of eels are found to be very rich in this enzyme or pump.

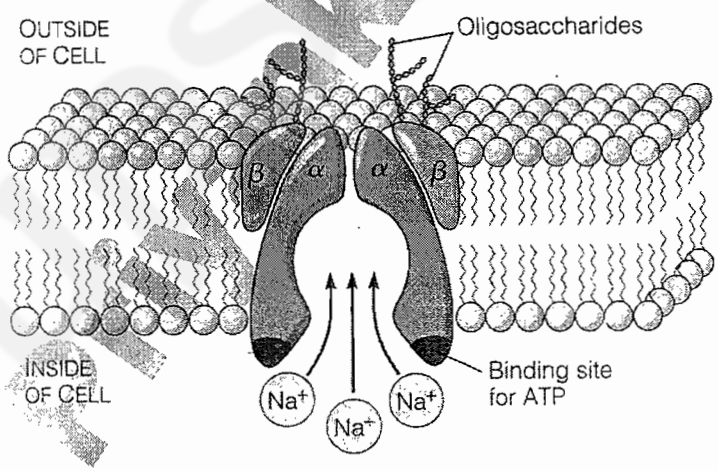


FIGURE 3: The organization of the Na^+/K^+ pump

There are four different types of ATP driven pump families for the different types of ions or molecules such as calcium pump, proton pump, etc. The diversity of ATP dependent pumps is summarized in Table 2.

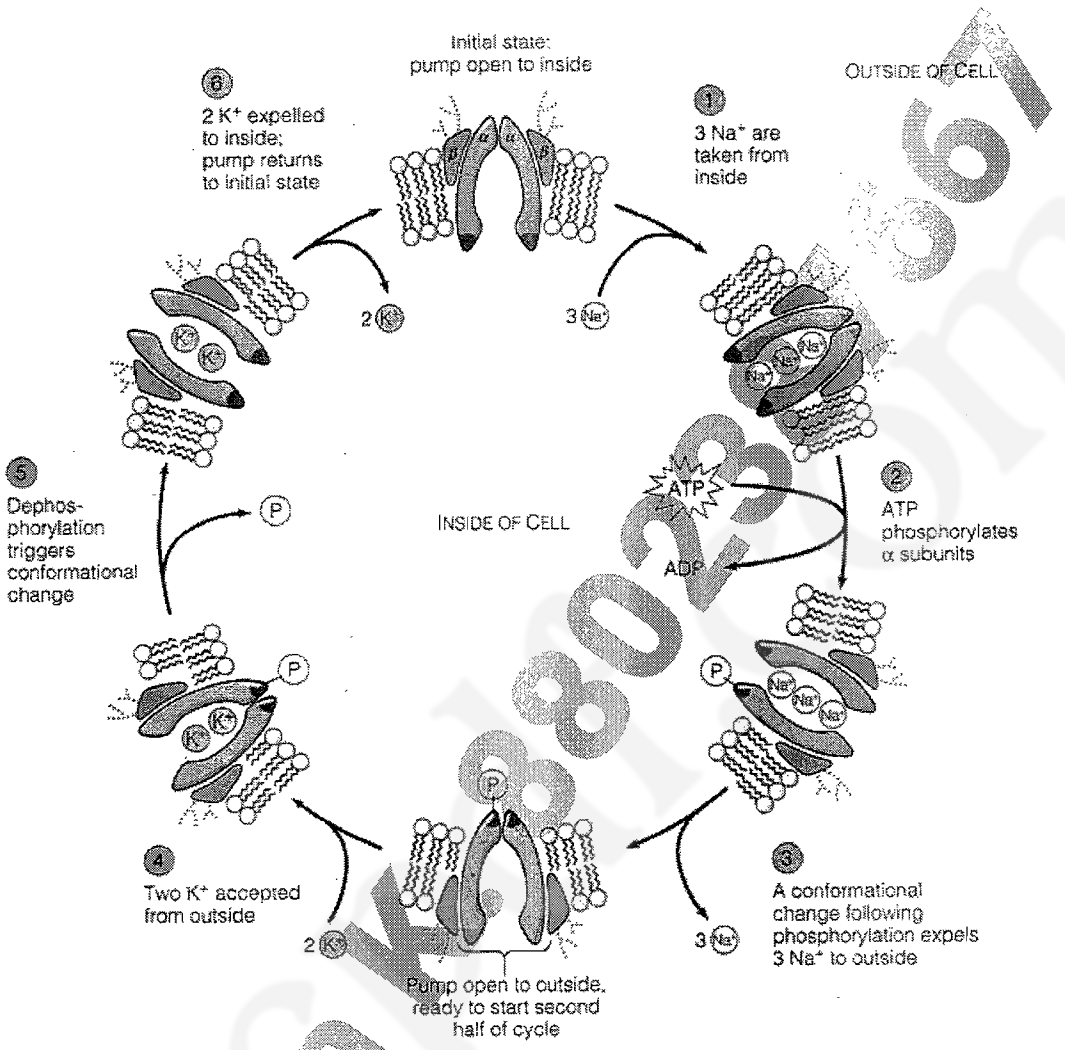


FIGURE 4: The working of the Na^+/K^+ pump

Table 2. ATP-DRIVEN TRANSPORT ATPase PUMPS

Pump	Subunits	Distribution	Substrate	Function
F_0F_1 Family				
F_0F_1	8 or more	Mitochondria, chloroplasts, bacterial, plasma membranes	H^+ (rarely Na^+)	ATP synthesis or ATP-driven H^+ pumping
V type ATPase Family				
V_0V_1	8 or more	Eukaryotic endomembranes, Archaea	H^+ (rarely, Na^+)	ATP-driven H^+ (or rarely, Na^+) pumping

P-type ATPase Family

Na^+K^+-ATPase	2	Plasma membrane	3 Na^+ for 2 K^+	Generation of Na^+, K^+ gradient
H^+K^+-ATPase	2	Stomach and kidney plasma membranes	1 H^+ for 1 K^+	Gastric and renal H^+ secretion
SERCA Ca-ATPase	1	Sarcoplasmic reticulum, endoplasmic reticulum	2 Ca^{2+} for 2 H^+	Lowering of cytoplasmic Ca^{2+}
PMCA Ca-ATPase	1	Plasma membrane	1 Ca^{2+} for 1 H^+	Lowering of cytoplasmic Ca^{2+}
H^+-ATPase	1	Plasma membrane in yeast, plants, protozoa	1 H^+	Generation of proton gradient

ABC Transporters

MDR1 P-glycoprotein	1	Plasma membrane	Drugs	Drug secretion
CFTR	1	Respiratory tract and pancreatic epithelial plasma membranes	ATP, Cl^-	Cl^- secretion
TAP1, 2	2	Endoplasmic reticulum	Antigenic peptides	Transport of antigenic peptides from cytoplasm into ER
MDR2	1	Liver cell apical plasma membrane	Phosphatidylcholine	Phosphoglyceride flippase, bile secretion?
STE6	1	Yeast plasma membrane	Mating pheromone peptide	Signaling for mating
HisQMP	4 + pp	Bacteria plasma membrane	Histidine	Histidine uptake
PstSCAB	4 + pp	Bacteria plasma membrane	Phosphate	Phosphate uptake
OppDFBCA	4 + pp	Bacteria plasma membrane	Oligopeptides	Peptide uptake
HlyB	2	*Escherichia coli* plasma membrane	Hemolysin A (107-kD protein)	Hemolysin A uptake

The ABC Transporters in Active Transport

ABC ("ATP-**B**inding **C**assette") transporters are transmembrane proteins that expose a ligand-binding domain at one surface and a ATP-binding domain at the other surface. The ligand-binding domain is usually restricted to a single type of molecule. The ATP bound to its domain provides the energy to pump the ligand across the membrane. The human genome contains 48 genes for ABC transporters. Some examples:

- **CFTR**— the cystic fibrosis transmembrane conductance regulator
- **TAP**, the transporter associated with antigen processing.
- The transporter that liver cells use to pump the salts of bile acids out into the bile.

- ABC transporters that pump chemotherapeutic drugs out of cancer cells thus reducing their effectiveness.

ABC transporters must have evolved early in the history of life. The ATP-binding domains in archaea, eubacteria, plants and animals all share a homologous structure, the ATP-binding "cassette". In plants ABC Transporters carry out the uptake of Nitrates, a source of an essential nutrient.

Secondary Active Transport

In the classical pump function, the hydrolysis of ATP and transport of the substrate are directly coupled events. However, there are some active transport processes, in which ATP hydrolysis is utilized to create a specific type of ionic gradient. Once such an ionic gradient is created, it drives the passive flux of the actual substrate meant to be transported. S-GLUT is a well-studied example in animals. Its mode of action is shown in Figure 5. First of all, the Na^+/K^+ pump extrudes sodium ions in the exoplasm. Once sufficient concentration of sodium ions is built in the exoplasm, it drives transporter mediated uptake of glucose.

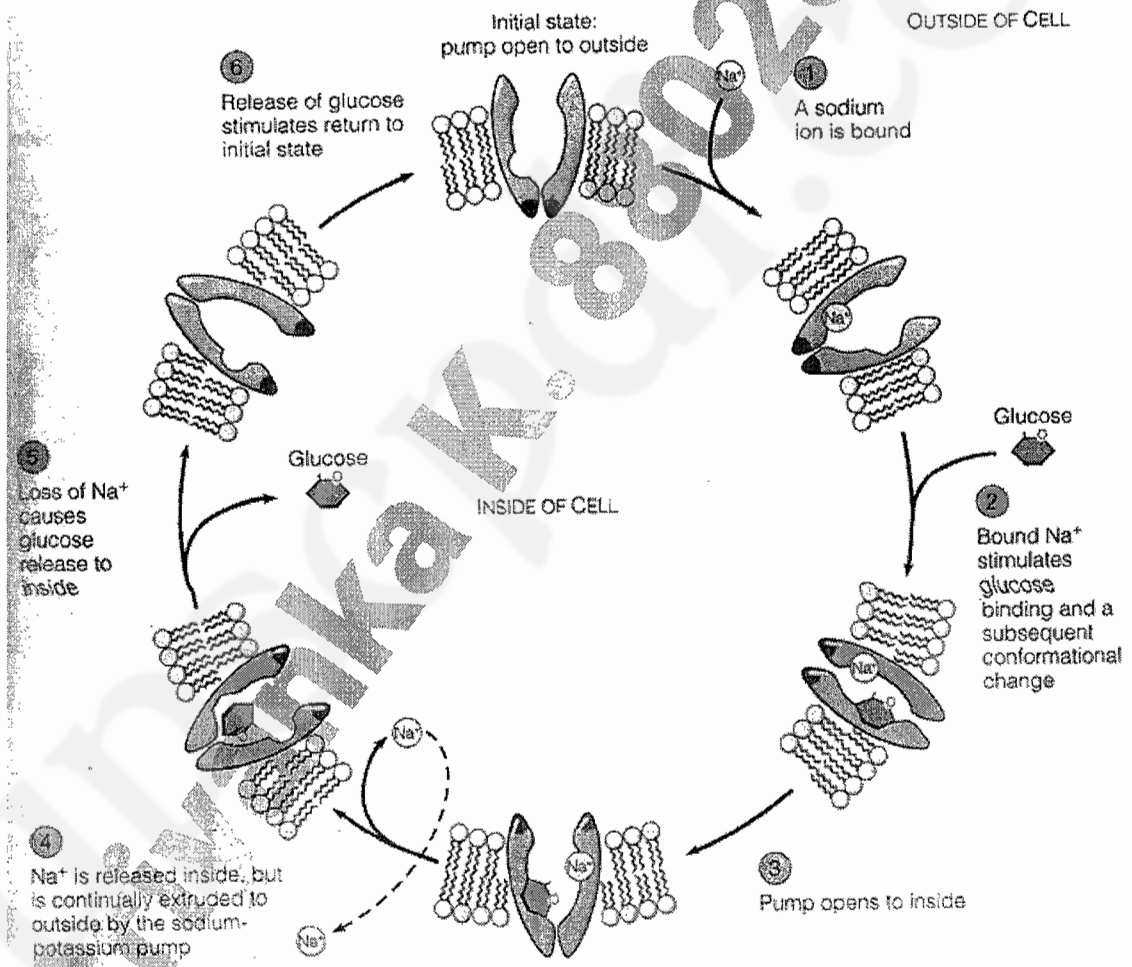
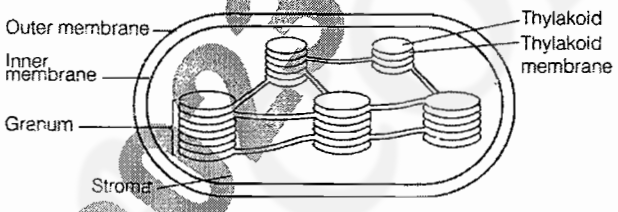


FIGURE 5: The functioning of S-GLUT

CHLOROPLAST

Introduction to the Plastids & Chloroplasts

Plastids are characteristic of plant cells and otherwise only occur in plant-like protists like the Euglenoids. They are generally round, oval, or disc-shaped bodies about 4 to 6 micrometer in diameter and observable under the light microscope. Two unit membranes, called an envelope, are at the surface. Internally, plastids consist of a membrane system and matrix. Proplastids give rise to plastids. There are several types of plastid in plant cells.



Chloroplasts are photosynthetic plastids found in the mesophyll cells of leaves, the cortex of herbaceous stems and in small numbers elsewhere in the plant. The green coloration is due to the presence of the pigment chlorophyll. Chloroplasts are bounded by a double-membrane envelope, with the internal structure consisting of membranes and the nonmembrane area, or *stroma*. In the chloroplast, there is an elaborate structure of membranes that resemble simple, flattened sacs called *stroma lamellae*. Other membranes are more concentrated in areas of the chloroplast and form stacks of disklike, flattened sacs called *thylakoids*. *Grana* (singular: granum) are collections of 5 to 50 thylakoids and appear as stacks of miniature pancakes. The *grana thylakoids* are often connected to the stroma lamellae. Chloroplasts are highly organized for photosynthesis. The photosynthetic pigments are arranged in the stacks so that they can be orientated to capture as much light energy as possible.

As with the mitochondria, chloroplasts (and plastids in general) contain DNA and RNA, the latter often seen as 70S ribosomal particles. The plastid genome is circular, dsDNA like the prokaryotic chromosomes. This, together with the presence of the double outer membrane, has led to suggestions that they arose as endosymbionts – primitive photosynthetic organisms that colonized a non-photosynthetic cell. However, while some chloroplast proteins are synthesized on plastid ribosomes (70S), from genes in the chloroplast genome, many others are encoded by nuclear genes and imported.

Chromoplasts contain pigments other than chlorophyll and are associated with brightly colored structures like ripe fruit. Chromoplasts are plastids that contain carotenoid pigments only. The function of chromoplasts is obscure, but they are responsible for the coloring of autumn leaves, flowers, and fruit. In ripening fruit or fruit peel, for example, the internal membrane structure and chlorophyll of the chloroplasts is lost while carotenoids accumulate to form the chromoplasts. A familiar example of the conversion of chloroplasts to chromoplasts is in ripening tomato berries.

Leucoplasts are colorless and are found in many cell types. Leucoplasts are nonpigmented plastids, devoid of chlorophyll and carotenoids, and are prevalent in cells of certain plant organs, including leaves, roots, and storage organs. They include **amyloplasts** that store starch and **elaioplasts** that synthesize lipid. (When plastids play an extensive role in starch biosynthesis, as in the cells of potato

tubers and the endosperm of corn kernels, they are termed amyloplasts.) Leucoplasts, which also produce proteins, oils, and other substances, can develop chlorophyll and become chloroplasts upon exposure to light.

Etioplasts are an intermediate stage in the production of photosynthetic chloroplasts in tissue exposed to light for the first time.

Chloroplasts

Overview

Chloroplasts are double membrane enveloped, about 5 μm long organelles found in plant cells and eukaryotic algae that conduct photosynthesis. Chloroplasts absorb sunlight and use it in conjunction with water and carbon dioxide to produce sugars. Chloroplasts capture light energy from the sun to conserve free energy in the form of ATP and reduce NADP to NADPH through a complex set of processes called photosynthesis.

Chloroplasts perform the entire primary (e.g. light capture and electron transport leading to NADPH and ATP synthesis) and most of the secondary processes (e.g. synthesis of 3-carbon phosphorylated compounds from CO_2) of photosynthesis. They also synthesize many proteins and other components.

Chloroplasts not only contain all the membrane-bound light-harvesting chlorophyll and other pigments, proteins and redox compounds involved in transport of electrons and synthesis of ATP but they also contain the soluble enzymes and substrates required for CO_2 and NO_3 assimilation by photosynthesis together with its products.

Evolutionary Origin

Chloroplasts are one of the many unique organelles in the cell, and are generally considered to have originated as endosymbiotic cyanobacteria. In this respect they are similar to mitochondria, but are found only in plants and protista. Both organelles are surrounded by a double layered composite membrane with an intermembrane space; both have their own DNA and are involved in energy metabolism; and both have reticulations, or many infoldings, filling their inner spaces.

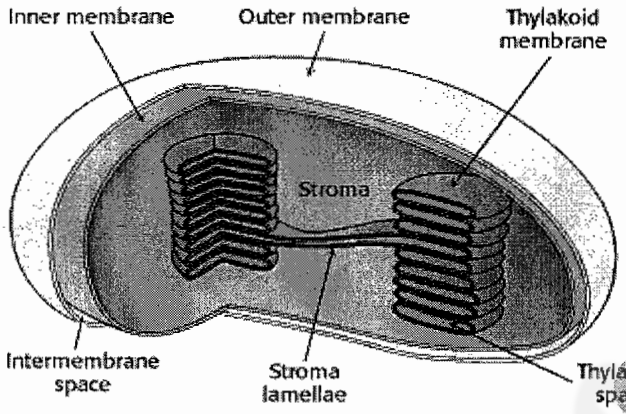
In green plants, chloroplasts are surrounded by two lipid-bilayer membranes. The inner membrane is now believed to correspond to the outer membrane of the ancestral cyanobacterium. The chloroplast genome is considerably reduced compared to that of free-living cyanobacteria, but the parts that are still present show clear similarities. Many of the missing genes are encoded in the nuclear genome of the host. (*Please refer to a later discussion on Chloroplast Genome*).

In some algae (such as the heterokonts and other protists such as Euglenozoa and Cercozoa), chloroplasts seem to have evolved through a secondary event of endosymbiosis, in which a eukaryotic cell engulfed a second eukaryotic cell containing chloroplasts, forming chloroplasts with three or four membrane layers.

Structure: Overview

Chloroplasts are observable morphologically as flat discs usually 2 to 10 micrometer in diameter and 1 micrometer thick. The chloroplast has a two-membrane envelope termed the Inner & Outer membrane respectively. Between these two layers is the intermembrane space.

The gel state material within the chloroplast is called the stroma, which contains one or more molecules of small circular DNA. It also contains 70S ribosomes, although most of its proteins are encoded by genes contained in the cell nucleus, with the protein products transported to the chloroplast. The stroma contains soluble enzymes and substrates required for CO_2 and NO_3 assimilation by photosynthesis together with its products.



Within the stroma are stacks of thylakoids, the sub-organelles where photosynthesis actually takes place. A stack of thylakoids is called a granum (plural: grana). A thylakoid looks like a flattened disk, and inside is an empty area called the thylakoid space or lumen. The photosynthesis reaction takes place on the membrane of the thylakoid, and it involves the coupling of cross-membrane fluxes with biosynthesis via the dissipation of a proton electrochemical gradient.

Embedded in the thylakoid membrane is a dish like structure of chlorophyll molecules known as the antenna complex. This outer array helps to increase the surface area of light capture. The photons are then funneled to the centre of this complex. Two chlorophyll molecules are then ionised, producing an excited electron which then passes onto the photochemical reaction centre.

Structure: Detailed

The Envelope

Electron microscopy shows the chloroplast to consist of an envelope made up of two separate membranes, enclosing a complex of membranes, the thylakoid system. Two membranes form the envelope; each is about 5.6 nm thick and they are separated by the intra-envelope space (10 nm), with areas of high electron density between the membranes which are possibly contact points involved in transport, for example of proteins between cytosol and stroma. The membranes are lipid bilayers of galactolipids (some 75%) with very unsaturated fatty acids (galactosyl glycerides and phosphatidyl choline), containing carotenoids but no chlorophyll. Monogalactosyldiglyceride (MGDG) makes up 50% of the membranes.

The membranes are not identical in structure or function. The outer cytoplasmic membrane allows many substrates to pass freely, whereas the inner (stromal) membrane is highly selective, allowing passage of only -some solutes by special enzyme systems called translocators. Protein particles in both membranes are complexes associated with the transporters and also with transport of other proteins.

The Stroma

The chloroplast stroma is not a homogeneous aqueous solution of small molecules and dilute proteins. In electron micrographs it contains indistinct granules and particles, which are mainly proteins, since the stroma is a dense protein gel, with about 0.4 g protein cm^{-3}. The most abundant protein is ribulose bisphosphate carboxylase/ oxygenase (RUBISCO) which forms over half of the protein; in some conditions, such as water stress or air pollution, it may form crystals.

In addition to RUBISCO, there is a large concentration of RUBISCO Activase. Also, all the other enzymes of the photosynthetic carbon reduction cycle are in the stroma, together with the enzymes and terminal redox carriers of the electron transport chain.

ATP synthase also protrudes from the thylakoids into the stroma. Other inclusions are products of the photosynthetic processes; for example, starch granules accumulate in the stroma and displace the thylakoid membranes, and globules of lipids and plastoquinone accumulate, often markedly so under stress conditions or in old leaves.

The ds, circular DNA of the chloroplast genome and mRNA plus protein-synthesizing system (70S Ribosomes) also occur in chloroplast stroma which synthesize many of their constituent proteins.

The genome of the chloroplasts found in *Marchantia polymorpha* contains 121,024 base pairs in a closed circle. These make up some 128 genes which include:

- duplicate genes encoding each of the four subunits (23S, 16S, 4.5S, and 5S) of the **ribosomal RNA (rRNA)** used by the chloroplast
- 37 genes encoding all the transfer RNA (tRNA) molecules used for translation within the chloroplast. Some of these are represented in the figure by black bars (a few of which are labeled).
- 4 genes encoding some of the subunits of the RNA polymerase used for transcription within the chloroplast (3 of them shown in blue)
- a gene encoding the large subunit of the enzyme **RUBISCO** (ribulose bisphosphate carboxylase oxygenase)
- 9 genes for components of photosystems I and II
- 6 genes encoding parts of the chloroplast ATP synthase
- genes for 19 of the ~60 proteins used to construct the chloroplast ribosome

All these gene products are used within the chloroplast, but all the chloroplast structures also depend on proteins

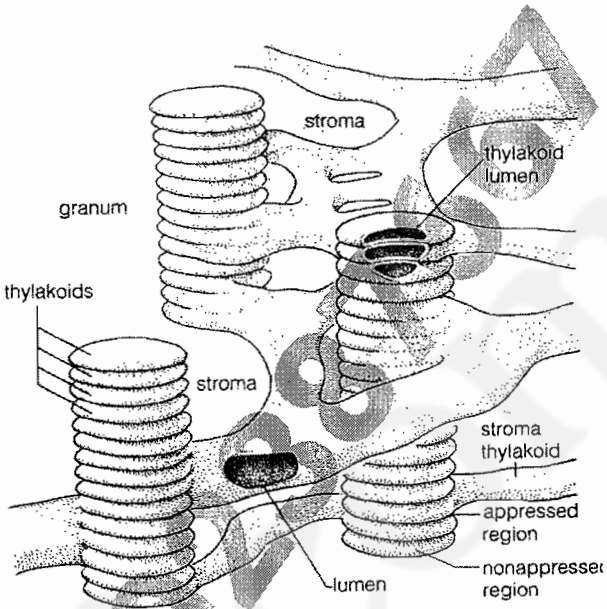
- encoded by nuclear genes
- translated in the cytosol, and
- imported into the chloroplast.

RUBISCO, for example, consists of multiple copies of two subunits:

- a large one encoded in the chloroplast genome and synthesized within the chloroplast, and
- a small subunit encoded in the nuclear genome and synthesized by ribosomes in the cytosol. The small subunit must then be imported into the chloroplast.

The Thylakoid System

The most noticeable features of chloroplasts in electron micrographs are the thylakoids, which are sack-like extensive membrane vesicle system. In transverse section the thylakoids appear as parallel pairs of continuous membranes separated by a space, the thylakoid lumen, which is 5-10 nm wide. Thylakoid membranes frequently associate into granal stacks, interconnected by pairs of membranes, called stromal thylakoids (also called intergranal connections or frets), which are in contact with the stroma on both sides. The interface between the granal thylakoids are called the appressed regions. In C3 plants over 60% of the thylakoid surface is typically in the granal organisation. The outer and end membranes of granal stacks and the stromal membranes, but not the appressed regions, have direct contact with the stroma.

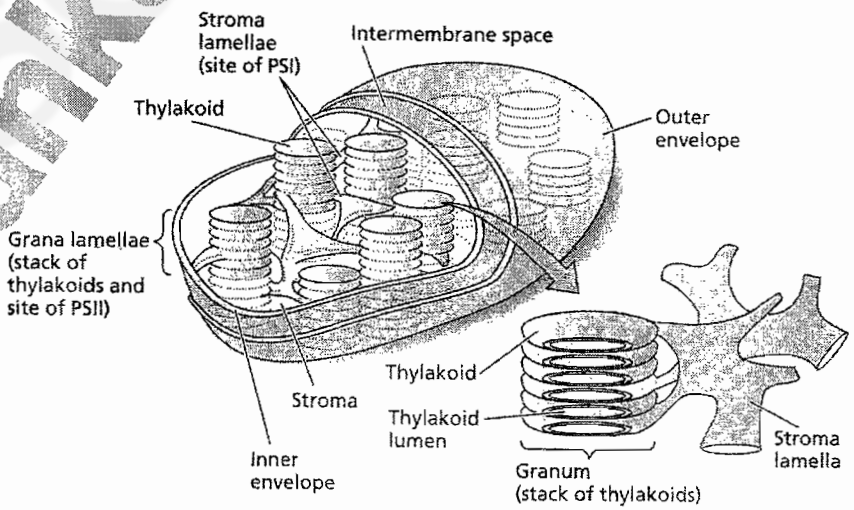


The thylakoid system appears to be a single interconnecting giant closed vesicle with continuous lumen, a feature of great importance in electron transport and ATP generation. It is dynamic, changing form and relative position within the chloroplast. This may be related to the movement of materials within the chloroplast.

Thylakoid membranes are constructed of lipid with many protein complexes embedded. The composition of the thylakoid lumen is not known, but proteins of the water-splitting complex and the light-harvesting complex for example, protrude from the membranes into the lumen and occupy part of the volume.

The thylakoid membrane is a single bilayer membrane is 5-7 nm thick and consists of lipid (30% of the mass) together with proteins, pigments and other major components which are vital for photosynthesis.

Thylakoid lipids are a complex mixture; some 80% is glycolipid containing galactose, such as MGDG diglyceride and digalactosyl-diglyceride (DGDG). DGDG is very important in photosynthetic membranes, and changing the



proportion of DGDG in thylakoids decreases PSII efficiency and energy transfer. The fatty acids of lipids are highly unsaturated. Linolenic acid (C18:3) is the predominant fatty acid.

Vitamin E (α-tocopherol) is a lipophilic constituent of the thylakoid membrane which may provide structure to the membrane and the protein complexes in it. However, α-tocopherol also has significant **photoprotective function**. Thylakoid membranes are subjected to intense radiation in an environment in which oxygen is produced, as well as highly energetic pigments, much reduced intermediates of electron transport and so on. They are therefore very liable to damage from, for example, reactive oxygen species, which may cause lipid peroxidation, thus destroying the membrane, or damaging protein complexes such as PSII by photoinhibition. α-tocopherol being an important antioxidant, prevents such damages.

Thylakoid membranes are particularly fluid compared with other membranes in plants; this is probably essential for the photosynthetic mechanism, with the abundant pigment-protein complexes moving within the lipid layers laterally and vertically, and also rotating. The lateral diffusion coefficient of lipids is 10^{-10} m^2 S^{-1} and that of proteins 5 X 10^{-11} m^2 S^{-1}. Distances over which pigment-protein complexes move are small (1-1000 nm) so displacements of the order of 10-100 nm occur rapidly, particularly if the proteins are charged.

Particles or Complexes in Thylakoid Membranes

The surface structure of thylakoid membranes is observed by electron microscopy of isolated membranes and internal structure after freeze-fracturing (by cutting) frozen membranes. Particle distribution on fractured membranes has been analyzed mainly on Spinach (*Spinacea*) and it shows large particles in two populations of 15 (Pigment System 2 or PS-II) and 11 nm diameter (Pigment System 1 or PS-I), 60-70% and 30-40%, respectively.

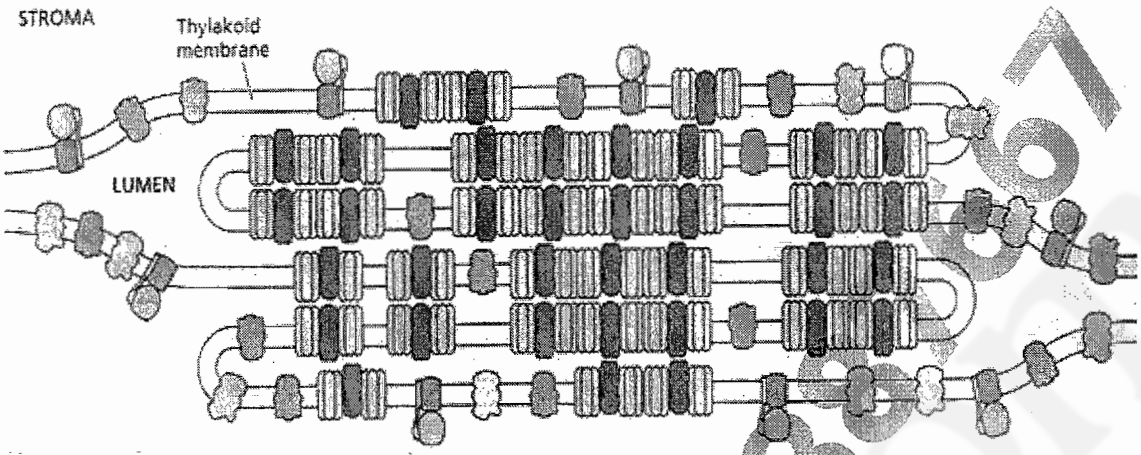
During analysis of the freeze-fractured thylakoid membranes, 4 prominent complexes are observed.

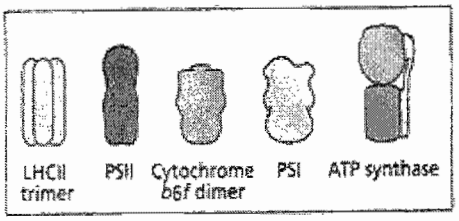
1. ATP synthase (CF1)
2. Pigment System 1 (PS-I) and the associated Light Harvesting Complex (LHC - I)
3. Pigment System 2 (PS-II) and the associated Light Harvesting Complex (LHC - II)
4. The Cytochrome b_6f complex: The *cytochrome* b_6f complex, are homologous to mitochondrial ubiquinol cytochrome *c* oxidoreductase. The cytochrome b_6f complex includes four subunits: a 23-kd cytochrome with two *b*-type hemes, a 20-kd Rieske-type Fe-S protein, a 33-kd cytochrome *f* with a *c*-type cytochrome, and a 17-kd chain.

These complexes are asymmetrically distributed, which is depicted below. This asymmetric distribution has a functional significance in the Light Reactions of Photosynthesis.

From the picture below, it is clear that:

- ATP synthase (CF1) is located mostly in stromal thylakoid.
- Pigment System 1 (PS-I) and the associated Light Harvesting Complex (LHC - I) are located mostly in stromal thylakoid.
- Pigment System 2 (PS-II) and the associated Light Harvesting Complex (LHC - II) are located mostly in granal thylakoid's appressed region.
- The Cytochrome b_6f complex is uniformly distributed.





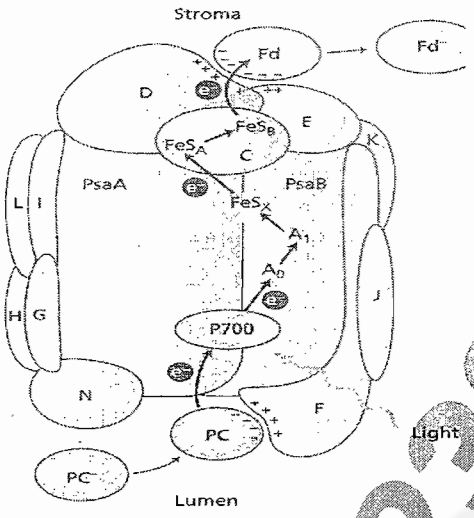
The Photosystem 1

PSI is almost restricted to membranes exposed to the stroma and absent from the interior of stacked membranes. The final stage of the light reactions is catalyzed by photosystem I.

Photosystem I typically includes 13 polypeptide chains, more than 60 chlorophyll molecules, a quinone (vitamin K_1), and three 4Fe-4S clusters. The total molecular mass is more than 800 kd. The core of this system is a pair of similar subunits psaA (83 kd) and psaB (82 kd). These subunits are quite a bit larger than the core subunits of photosystem II. A special pair of chlorophyll *a* molecules lies at the center of the structure and absorb light maximally at 700 nm. This center, *P700*, initiates photoinduced charge separation.

The PSI core is now called CPC I (also called P700 chlorophyll *a* complex or chlorophyllprotein complex I, CPC I for short) and is associated with a light-harvesting chlorophyll a/b-protein complex, now called light-harvesting complex or LHCI, composed of four different types of complexes (a-d), which has only antenna function and no photochemical activity.

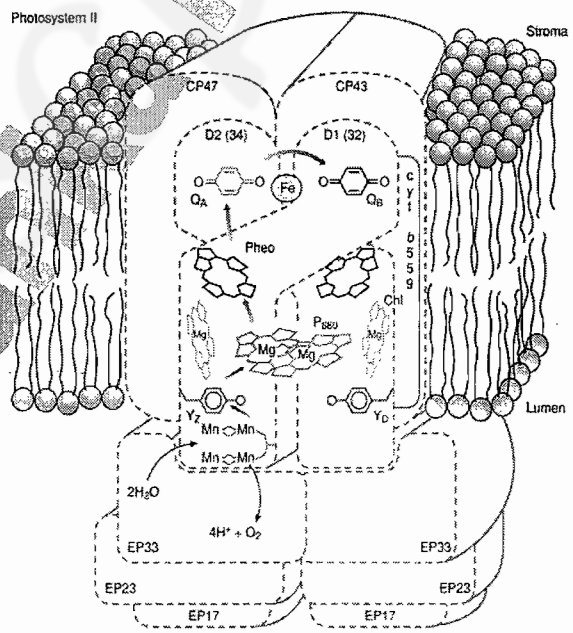
The structure of PS I is depicted below. The diagram also shows the flow of electrons through the PS I.



The Photosystem 2

The PSII complex occurs in all oxygen-evolving plants (and is thus essential for 02 evolution) and contains 10% of the total chlorophyll, mainly or only chlorophyll *a* for the inner or close antenna. Probably 80% of PSII is in the appressed granal regions, away from the stromal thylakoids.

Photosystem II of green plants is reasonably similar to the bacterial reaction center. The core of photosystem II is formed by D1 and D2, a pair of similar 32-kd subunits that span the thylakoid membrane. These subunits are homologous to the L and M chains of the bacterial reaction center. Unlike the bacterial system, photosystem II contains a large number of additional subunits that bind additional chlorophylls and increase the efficiency with which light energy is absorbed and transferred to the reaction center



The PSII complex has a core of CPC II (chlorophyllprotein complex II, CPC II for short), which is probably a dimer of 230 kDa mass, each composed of two polypeptides of 50 or 60 and 70 kDa, and contains about 30-40% of the total chlorophyll *a* (no chl *b*). Photosystem II is slightly less complex but larger than the PS I. The entire PS II at least 10 polypeptide chains, more than 30 chlorophyll molecules, a nonheme iron ion, and four manganese ions.

The photochemistry of photosystem II begins with excitation of a special pair of chlorophyll molecules that are bound by the D1 and D2 subunits. This pair of molecules is analogous to the special pair in the bacterial reaction center, but it absorbs light at shorter wavelengths (maximum absorbance at 680 nm) because it consists of chlorophyll *a* molecules rather than bacteriochlorophyll. The special pair is often called *P680*. The energy from the light excites an electron from its ground energy level to an excited energy level.

The structure of PS II is depicted hrere. The diagram also shows the flow of electrons through the PS II.

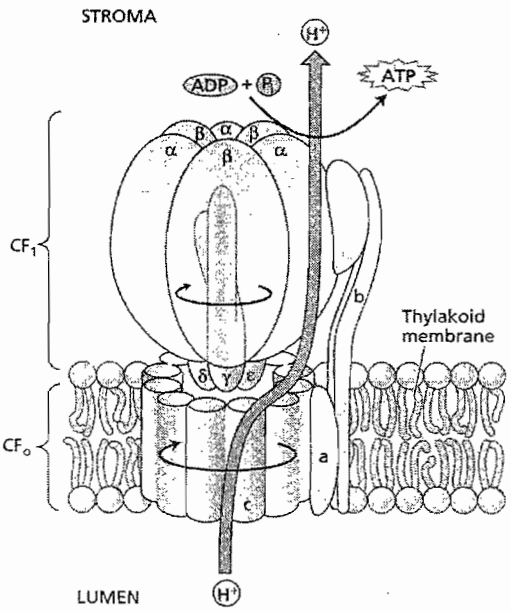
The Plastidial ATP Synthase Complex

The proton-motive force generated by the light reactions is converted into ATP by the *ATP synthase* of chloroplasts, also called the *CF_1-CF_0complex* (*C* stands for chloroplast and *F* for factor). CF_1-CF_0 ATP synthase closely resembles the F_1-F_0 complex of mitochondria.

CF_0 conducts protons across the thylakoid membrane, whereas CF_1 catalyzes the formation of ATP from ADP and P_i.

CF_0 is embedded in the thylakoid membrane. It consists of four different polypeptide chains known as I (17 kd), II (16.5 kd), III (8 kd), and IV (27 kd) having an estimated stoichiometry of 1:2:12:1. Subunits I, II, and III correspond to subunits **a, b,** and **c,** respectively, of the mitochondrial F_0 subunit, and subunit IV is similar in sequence to subunit **a.** CF_1, the site of ATP synthesis, has a subunit composition $\alpha_3\beta_3\gamma\delta\varepsilon$. The β subunits contain the catalytic sites, similar to the F_1 subunit of mitochondrial ATP synthase.

The membrane orientation of CF_1-CF_0 is reversed compared with that of the mitochondrial ATP synthase. Thus, protons flow *out* of the thylakoid lumen through ATP synthase into the stroma. Because CF_1 is on the stromal surface of the thylakoid membrane, the newly synthesized ATP is released directly into the stromal space.



Functions of chloroplast

1. The main functions of the chloroplast is its role in photosynthesis, the process by which light is transformed into chemical energy, to subsequently produce food in the form of sugars. Water (H_2O) and carbon dioxide (CO_2) are used in photosynthesis, and sugar and oxygen (O_2) is made, using light energy. Photosynthesis is divided into two stages—the light reactions taking place in the thylakoid system, where water is split to produce oxygen,

and the dark reactions taking place in the stroma, or Calvin cycle, which builds sugar molecules from carbon dioxide. The two phases are linked by the energy carriers adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate ($NADP^+$).

2. Like mitochondria, chloroplasts use the potential energy stored in an H^+, or hydrogen ion gradient to generate ATP energy. The two photosystems capture light energy to energize electrons taken from water, and release them down an electron transport chain. ATP synthase uses this energy to phosphorylate adenosine diphosphate into adenosine triphosphate, or ATP.

3. Chloroplasts alone make almost all of a plant cell's amino acids in their stroma except the sulfur-containing ones like cysteine and methionine.

4. Chloroplasts make all of a cell's purines and pyrimidines—the nitrogenous bases found in DNA and RNA.

5. They also convert nitrite (NO_2^-) into ammonia (NH_3) which supplies the plant with nitrogen to make its amino acids and nucleotides.

6. Chloroplasts are the site of starch synthesis.

7. Chloroplasts are the site of complex lipid metabolism.

8. Chloroplasts, along with the nucleus, cell membrane, and endoplasmic reticulum, are key players in pathogen defense. Plants have two main immune responses—the hypersensitive response, in which infected cells seal themselves off and undergo programmed cell death, and systemic acquired resistance, where infected cells release signals warning the rest of the plant of a pathogen's presence. Chloroplasts stimulate both responses by purposely damaging their photosynthetic system, producing reactive oxygen species. High levels of reactive oxygen species will cause the hypersensitive response. The reactive oxygen species also directly kill any pathogens within the cell. Lower levels of reactive oxygen species initiate systemic acquired resistance, triggering defense-molecule production in the rest of the plant.

9. Chloroplasts can serve as cellular sensors. After detecting stress in a cell, which might be due to a pathogen, chloroplasts begin producing molecules like salicylic acid, jasmonic acid, nitric oxide and reactive oxygen species which can serve as defense-signals.

MITOCHONDRIA

Introduction

A **mitochondrion** (plural **mitochondria**) is a double membrane-enclosed (enveloped), 1—4 micrometers (μm) long, thread or rod shaped cytosolic organelle found in most eukaryotic cells, which gets described as *cellular power house* because by oxidative breakdown of food molecules it generates most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as cellular differentiation, cell death, as well as the control of the cell cycle and cell growth. The Mitochondria are genetically semi-autonomous eukaryotic cell organelles with an endosymbiotic origin (Lynn Margulis, 1980).

Distribution and localization

The mitochondria move autonomously in the cytoplasm, so they generally have uniform distribution in the cytoplasm, but in many cells, their distribution is very restricted. The distribution and number of mitochondria (and of mitochondrial cristae) are often correlated with type of function the cell performs. Essentially, the number of mitochondria per cells varies depending on the energy requirements. *A large number of mitochondria (and, usually with many cristae) are associated with mechanical and osmotic work situations, where there are sustained demands for ATP e.g.,*

1. along the pseudopodia of *Amoeba* spp.,
2. between muscle fibres,
3. in the basal in-folding of kidney tubule cells,
4. in the peripheral regions of fungal haustoria (where rapid nutrient absorption from the host cell is taking place), and
5. in a portion of inner segment of rod and cone cells of retina.

The cells of green plants contain a lesser number of mitochondria in comparison to animal cells because in plant cells the ATP generating function of mitochondria, at least partially, is taken over by the chloroplasts.

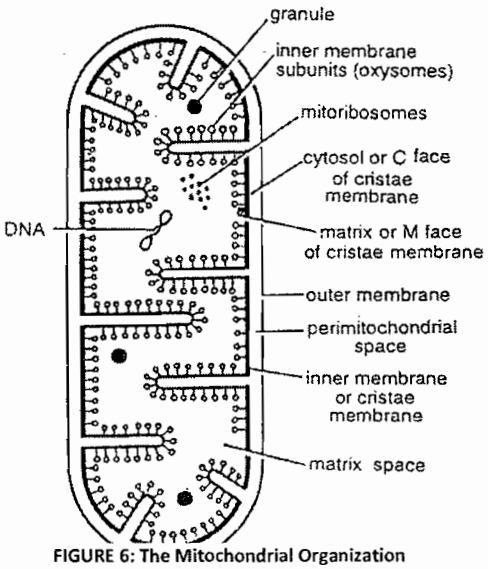


FIGURE 6: The Mitochondrial Organization

Structure, chemical composition & ultra structure

Gross Structure

The mitochondria are generally filamentous or granular in appearance; however, it varies depending upon the type and physiological conditions of the cells.

Mitochondria are about 0.5–1 μm in diameter and mostly between 1 μm and 4 μm in length. In some cells, the length may go up to 7 μm. Their shape and number per cell depends on the particular tissue. They may appear as spheres, rods or filamentous bodies, but the general architecture is the same (Figure 1).

In some cells, especially those with high energy demands, the organization of the mitochondria may be very different. For example, three-dimensional models of mitochondria constructed by **Hans-Peter Hoffmann** and **Charlotte J. Avers** from 80 to 150 consecutive serial sections of entire yeast cells showed that all the separate mitochondrial profiles were cross sections through a single, branching, tubular structure about 50 to 60 micrometers in length and 200 to 600 nanometers in diameter. The data are contrary to conventional notions of mitochondrial size, form, and number per cell.

Fine Structure

Mitochondria have two membranes, each composed of a phospholipid bilayer. The two membranes are quite distinct in appearance and in physico-chemical properties, thus determining the biochemical function of each membrane. Each of the mitochondrial membrane is 7-8 nm in thickness and fluid-mosaic in ultra structure.

The outer membrane is smooth, i.e. without any regularly attached particles and has many copies of transport protein families, **translocons** and **porins**. The porins form large aqueous channels through the lipid bilayer, permeable to all molecules of MW up to 10KD.

Inside and separated from the outer membrane by a 6-8 nm wide **perimitochondrial space** is present the highly convoluted inner membrane. The convolutions form a series of infoldings, called **cristae**, in the matrix space. The inner mitochondrial membrane is much less permeable to ions and small molecules than the outer membrane, therefore providing compartmentalization through separation of the matrix from the cytosolic environment. This compartmentalization is a central feature of the conversion of free energy derived from oxidizable substrates. The inner mitochondrial membrane is, in fact, an electrical insulator and chemical barrier.

So, mitochondria are double membrane enveloped organelles in which the inner membrane divides the mitochondrial space into two distinct chambers: 1. the outer compartment, peri-mitochondrial space and 2. The inner compartment, or matrix space, which is filled with a gel-like proteinaceous material, called **mitochondrial matrix**. The mitochondrial matrix contains lipids, proteins, **circular DNA molecules, 55 S ribosomes.**

As a matter of rule, the cristae of higher plant mitochondria are tubular, while those of animal mitochondria are lamellar or plate-like.

Repeated units of stalked particles are attached at the interval of 10 nm to the matrical face of inner mitochondrial membrane, designated as **F0 –F1 particles**, which are meant for ATP synthesis. Recent estimates suggest that there are 10^4 to 10^6 elementary particles per mammalian mitochondrion. The ATP synthase, or F1F0-ATPase, utilizes the proton motive force to convert ADP and phosphate to ATP, thereby coupling electron transport and proton pumping to ATP synthesis. This multisubunit transmembrane protein, which is the most complex structure in the inner mitochondrial membrane, has attracted considerable experimental attention in recent years. In 1997, Paul Boyer and John Walker were awarded the Nobel Prize for their elucidation of the enzymatic mechanism underlying the synthesis of ATP. Apart from the F0 –F1 particles, there are four electron transport complexes embedded in the inner mitochondrial membrane as well, which are summarized below.

Enzyme complex	Mass (kd)	Subunits	Prosthetic group	Takes e⁻ from	Gives e⁻ to
Complex I **NADH-Q oxidoreductase**	880 600 in Plants	≥34	FMN Fe-S	NADH	Quinone
Complex II **Succinate-Q reductase**	140 125 in Plants	4	FAD Fe-S	$FADH_2$	Quinone
Complex III **Q-cytochrome c oxidoreductase**	250 500 in Plants	10 -11	Heme b_H Heme b_L Heme c_1 Fe-S	Quinone	CytC
Complex IV **Cytochrome c oxidase**	160 125 in Plants	10	Heme *a* Heme a_3 Cu_A and Cu_B	CytC	O_2

Physiological Significance of Mitochondrial Fine Structure

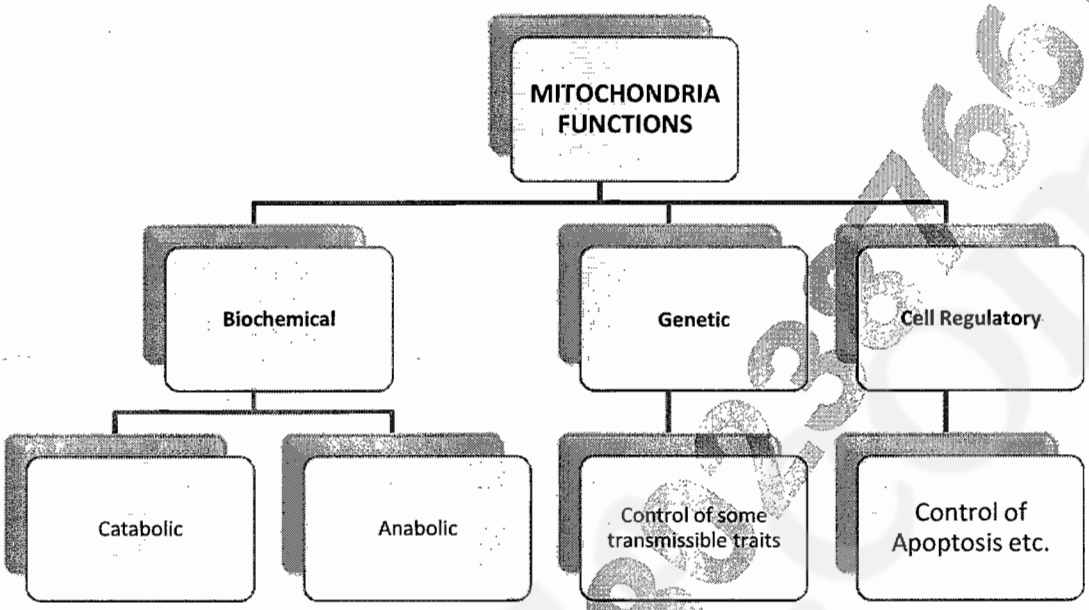
Mitochondria play a central role in energy metabolism of cells. They usually provide most of the ATP by oxidative phosphorylation. A major consequence of the architecture of mitochondria is the impermeability of the inner membrane that facilitates the generation of a proton gradient, called the proton motive force. The oxidative processes cells use to degrade fuel molecules yield NADH and FADH2 which are used as electron donors for the electron transport chain. The components of the chain are located in the inner mitochondrial membrane and include four complexes and some electron carriers. While electrons are transported along the chain, three of the four complexes act as proton pumps, expel protons from the matrix and build up the proton motive force. Another enzyme, the ATPase, utilizes the proton gradient to form ATP from ADP and Pi, thus allowing the protons to return to the matrix. The coupling of electron transport (i.e. oxidative processes) and ATP synthesis via the proton gradient is the main postulate of the chemiosmotic theory.

Due to the impermeability of the inner mitochondrial membrane to most solutes, a range of transporters exists which allow exchange of ions and metabolites (mostly in anionic form) between matrix and cytosol. These transporters also help to integrate mitochondrial and cytosolic metabolic pathways.

Mitochondrial Function

The mitochondria are multifunctional organelle of eukaryotic cells, though their primary role is carrying out aerobic respiration and generation of ATP, due to which they are also called the ***Power House of the Cell***.

The mitochondrial functions can be categorized in the following manner.



Biochemical functions of mitochondria

Mitochondrial biochemical functions are mainly catabolic but the mitochondrial processes generate a number of intermediates which can be used as precursors for several biosynthetic pathways. Accordingly the mitochondrial biochemical functions can be categorized as **catabolic** and **anabolic**.

Catabolic Functions

1. **PDH Reaction**: In an aerobic cell, when the ATP level is low, pyruvate generated during the glycolysis is preferentially metabolized via the the pyruvate dehydrogenase complex (PDH complex). This leads to the generation of Acetyl Co-A, which acts as the donor of acetyl group for the TCA cycle. The PDH reaction is as follows.
   Pyruvate + CoA + NAD+ → CO_2 + Acetyl-CoA + NADH + H^+
   This reaction is also called as the ***link reaction***, as it serves to link the Glycolytic process to the TCA pathway.

2. **TCA Cycle**: The **tricarboxylic acid cycle** (**TCA cycle**) is also called the **Krebs cycle**. It is a series of eight reactions taking place in the matrix of the mitochondria. In this cycle, two reduced carbon atoms are accepted from Acetyl CoA and then fully oxidized. In this process two CO_2 molecules are released and large amount of free energy is liberated. This free energy is conserved in form of 3 molecules of NADH, one molecule of $FADH_2$ and one molecule of GTP or ATP. For every glucose molecule being respired, the TCA cycle operated twice. The free energy conserved in form of NADH and $FADH_2$ is later used to synthesize ATP inside the mitochondria using the oxidative phosphorylation pathway.

3. **Oxidative phosphorylation**: It is a metabolic pathway that uses energy released by the oxidation of nutrients to produce ATP. During oxidative phosphorylation, electrons are taken

from NADH and $FADH_2$ and then transferred to the terminal electron acceptor which is oxygen. These transfers are carried out by the series of respiratory complexes within mitochondria. They release energy which is used for building up a proton gradient across the inner mitochondrial membrane. It is this proton gradient, which is used to form ATP by chemiosmotic mechanism.

4. **Beta oxidation**: It is the process by which fatty acids, in the form of Acyl-CoA molecules, are broken down in mitochondria to generate Acetyl-CoA. Acetyl CoA is the entry molecule for the Citric Acid cycle. Beta oxidation also occurs in peroxisomes.

   The beta oxidation of fatty acids involve three stages:

   a. Activation of fatty acids in the cytosol

   b. Transport of fatty acids into mitochondria

   c. Beta oxidation proper in the mitochondrial matrix

5. **Photorespiration**: It is also called the Photosynthetic Carbon Oxidation (PCO) Pathway. It occurs in some plants. Photorespiration involves three organelles, namely Chloroplast, Peroxisome and Mitochondria. Inside the mitochondrial matrix, the decarboxylation step of photorespiration occurs.

Anabolic Functions

In aerobic organisms, the citric acid cycle is an amphibolic pathway, one that serves in both catabolic and anabolic processes. The anabolic role arises from the fact that the cycle provides precursors for many biosynthetic pathways. Important examples are as follows.

1. α-Ketoglutarate and oxaloacetate can serve as precursors of the amino acids aspartate and glutamate by simple transamination. Through aspartate and glutamate, the carbons of oxaloacetate and α-ketoglutarate are then used to build other amino acids, as well as purine and pyrimidine nucleotides.

2. α-ketoglutarate is utilized for synthesis of purines and pyrimidines.

3. Oxaloacetate is converted to glucose in gluconeogenesis.

4. Malate is converted to glucose in gluconeogenesis.

5. Succinyl-CoA is a central intermediate in the synthesis of the porphyrin ring of heme groups, which serve as oxygen carriers (in hemoglobin and myoglobin) and electron carriers (in cytochromes)

6. Citrate can be used in fatty acid and steroid synthesis.

Role of Mitochondria in Regulation of Cellular Functions

Mitochondria play a central role in many other cellular tasks, such as:

1. Apoptosis-programmed cell death

2. Regulation of the membrane potential

3. Calcium signaling (including calcium-evoked apoptosis)
4. Cellular proliferation regulation

Mitochondrial control of apoptosis

Mitochondrial proteins known as SMACs (second mitochondria-derived activator of caspases) are released into the cytosol during apoptosis. SMAC binds to inhibitor of apoptosis proteins (IAPs) and deactivates them, preventing the IAPs from arresting the apoptotic process and therefore allowing apoptosis to proceed.

Cytochrome c is also released from mitochondria during apoptosis. Once cytochrome c is released it binds with Apoptotic protease activating factor Apaf-1 and ATP, which then bind to *pro-caspase-9* to create a protein complex known as an apoptosome which promotes the late stages of apoptosis.

Mitochondrial genetic system and genetic role

The Genetics of Mitochondria

Mitochondrial genetic systems consist of DNA and the molecular machinery needed to replicate and express the genes contained in this DNA.

This machinery includes the DNA Polymerase, RNA Polymerase and Ribosomes. These all are prokaryotic type.

Mitochondrial DNA

Mitochondrial DNA, or **mtDNA** was discovered in the 1960s, initially through electron micrographs and later through chemical procedures.

Mitochondrial DNA molecules vary enormously in size, from about 6 kb in *Plasmodium* to 2500 kb in some of the flowering plants. Each mitochondrion contains several copies of the DNA.

Most mtDNA molecules are circular, but in some species, such as the alga *Chlamydomonas reinbarditii* and the ciliate *Paramecium aurelia*, they are linear.

When the mt DNA is small, it is usually G:C pair rich, but this does not apply when mtDNA is large as in some of the flowering plants.

The structure of mtDNA molecules have been studied by DNA sequencing. Animal mtDNA is small and compact. In human beings, the mtDNA is 16,659 base pairs long and contains 37 genes, including two that encode ribosomal RNAs, 22 that encode transfer RNAs, and 13 that encode polypeptides involved in oxidative phosphorylation. In mice, cattle, and frogs, the mtDNA is similar to that of human beings. Invertebrate mtDNA is about the same size as vertebrate mtDNA, but it has a somewhat different genetic organization. These differences have been caused by structural rearrangements of the genes within the circular mtDNA molecule.

Expression of Mitochondrial Genes

The simple mtDNA of vertebrates are organized into two large transcription units, each encoding the information of several genes.

Each mRNA is then translated into polypeptides, using the mitochondrial ribosomes and a combination of nuclear and ribosomal rRNAs.

mtRibosomes are prokaryotic type in having three types of ribosomal RNA but they have different sedimentation values, for example: among fungi, the Mt ribosome is 77S type, the Mt ribosome in mammals is of 55S type, in plants they are of 70S type and in insects, the Mt ribosome is of 60S type.

During translation inside mitochondria, there are certain violations of the universal genetic code. These are known as *Novel Genetic Code.* Some examples are listed below.

Organism	**Codon**	**Standard**	**Novel**
Mammalian	*AGA, AGG*	Arginine	Stop codon
	AUA	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
Invertebrates	*AGA, AGG*	Arginine	Serine
	AUA	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
Yeast	*AUA*	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
	CUA	Leucine	Threonine

Another peculiarity of plant mitochondrial gene expression is that many of the mtRNA transcripts undergo **editing**; that is, some of the nucleotides are changed after the transcript has been synthesized. The most frequent change is C to U, but occasionally U is changed to C. Thus, RNA editing alters the composition of codons in plant mitochondrial transcripts.

Interplay between Mitochondrial and Nuclear Gene Products

All mitochondrial gene products function solely within the mitochondrion. However, they alone do not control the entire range of mitochondrial functions. Many nuclear gene products are imported to mitochondria for several functions.

For example, mitochondrial ribosomes are constructed with ribosomal RNA transcribed from mitochondrial genes and with ribosomal proteins encoded by nuclear genes. Many of the polypeptides needed for aerobic metabolism are also synthesized in the cytosol.

Mutations in Mitochondrial DNA and Resulting Abnormalities (including Human Diseases)

1. In yeasts, *petite mutants* are associated with alterations in the structure of the mtDNA. These mutants form dwarf colonies of *Saccharomyces cerevisiae.*

2. *Poky* are the most famous of the mitochondrial mutants of *Neurospora*. These slow-growing, respiration-defective fungi have reduced numbers of ribosomes due to deletions in mtDNA that impair the synthesis of rRNA.

3. Recent research has demonstrated that several human diseases are caused by mitochondrial defects. One such disease is **Leber's hereditary optic neuropathy (LHON)**, a condition characterized by the sudden onset of blindness in adults. Another disorder caused by a mutation in the mtDNA is **Pearson marrow pancreas syndrome.** This disease, characterized by a loss of bone-marrow cells, during childhood, is frequently fatal. In addition to these, several other myopathies and neuropathies are associated with mitochondrial mutations.

4. In plants species such as Allium, cytoplasmic male sterility is the result of specific nuclear and mitochondrial genetic interactions.It is total or partial male sterility associated with the failure of plants to produce functional anthers, pollen, or male gametes.

DNA Bar coding

While traditional methods for classifying plants and animals demand great skill and time, examining a small portion of the DNA is more accurate, faster and easier. In 2003, Mark Stoeckle and Paul Hebert proposed that a segment of mitochondrial DNA can be used to distinguish animal species.

The taxonomic method that uses a short genetic marker in an organism's DNA to identify it is called **DNA Barcoding.**

In DNA Barcoding of animal species, a desirable locus is the mitochrondrial CO1 gene. It is based on the concept that mitochondrial DNA (mtDNA) has a fast mutation rate, which results in significant variation in mtDNA sequences between species and a comparatively small variance within species.

ENDOPLASMIC RETICULUM

Found only in the eukaryotic cells, the endoplasmic reticulum (ER) is a functionally versatile cell organelle, present as an anastomosing network of sacs (cisternae), tubules and vesicles enclosed by a continuous membrane which extends from the nuclear envelope (NE) throughout the cytoplasm. The ER is in physical continuity with the nuclear envelope and shares an intimate functional relation with the Golgi Apparatus (GA). Together, the ER, GA and the NE make the Endomembrane System of a eukaryotic cell.

The ER serves as a multifunctional cell organelle. It serves specialized functions in the cell, including synthesis and modification of the proteins being targeted by the secretory pathway, organelle biogenesis, storage (and release) of calcium ions, production of steroids, lipid metabolism, storage and production of glycogen, etc.

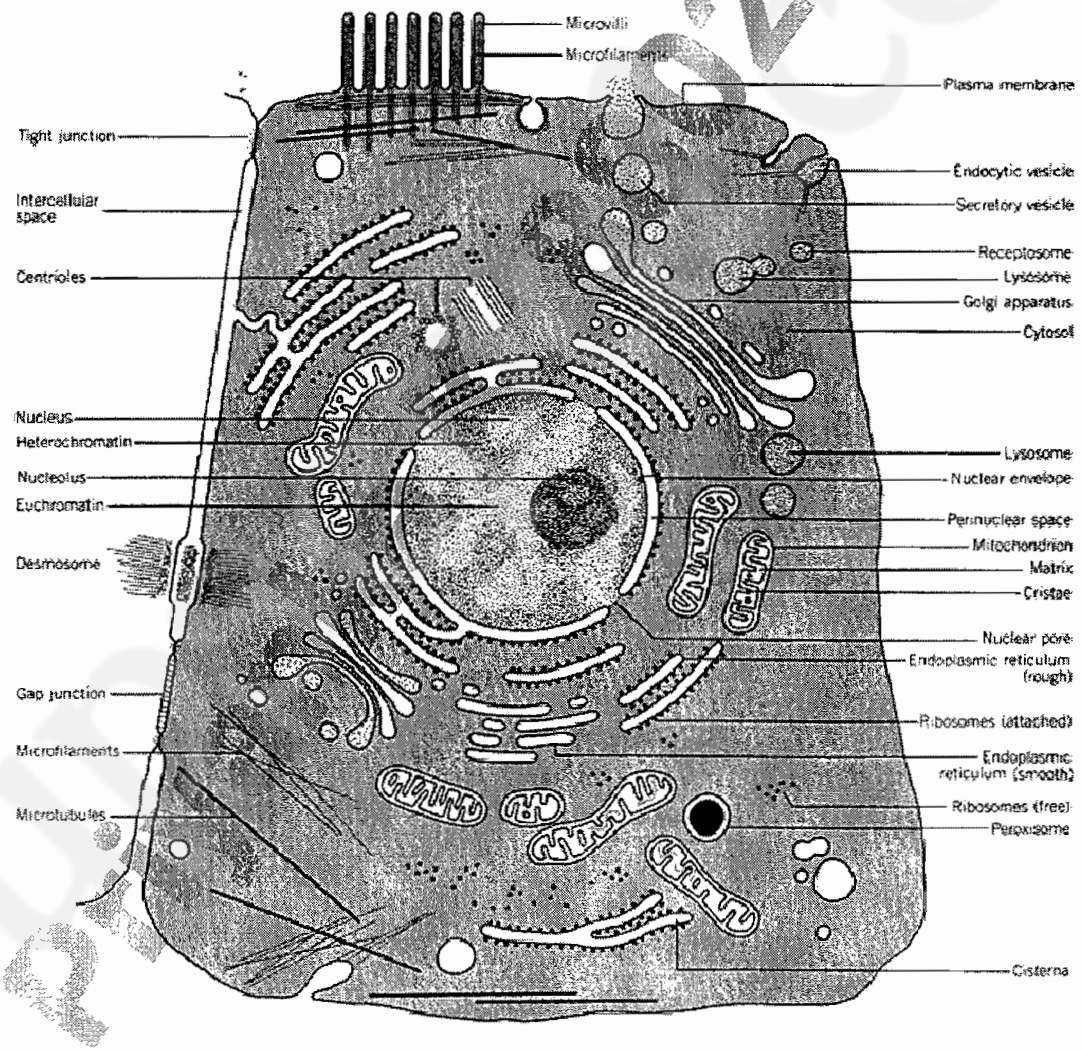


FIGURE 1: An animal cell showing the position of the ER

Distribution, Shape, Size and Relative Position in the Cell

The ER is found in all the eukaryotic cells, except some physiologically specialized cells such as mature RBCs of vertebrates, Sieve Element cells in plant phloem. Some cells in the early embryonic stages also lack the ER system.

The shape of the ER becomes apparent only in electron micrographs. Its appearance is like a series of interconnected flattened vesicles (cisternae) originating from the nuclear envelope. The margins are tubular and the overall structure is surrounded by some large uncoated vesicles.

The ER is usually the largest organelle of most animal cells. Its membrane may account for about half of all cell membranes, and the space enclosed by the ER (the lumen, or cisternal space) may represent about 10% of the total cell volume.

Within a cell, the ER is largely found around the nucleus. This is due to continuity between the ER and the nuclear envelope.

Relation with Other Cell Organelles

1. As shown in Figure 1, the ER remains in physical continuity with the nuclear envelope. For this reason, the nuclear envelope is also called the Perinuclear Cisterna.

2. In the process of protein targeting through the secretory pathway, the ER processes the nascent proteins in the initial stages, but only to some extent. The semi-processed protein then moves to the Golgi Apparatus (GA) for further processing and sorting. Thus, the ER and GA remain in biochemical and functional continuity. The semiprocessed proteins move from the ER to the GA through the Transition Vesicles.

3. All the cellular components which receive their proteins by the secretory pathway (namely the Plasma Membrane, Lysosomes, Secretory Vesicles and Endosomes) are functionally related to the ER in the sense that all their proteins have been primarily processed by the ER.

4. To highlight the relation of the ER with other cell organelles, the following three major concepts have been put forward.

   (i) **The GERL Concept,** given by Alexei Novikoff in late 1950s, in which he suggested that cellular secretion occurs by a process that involves Golgi Apparatus (G), Endoplasmic Reticulum (ER) and Lysosomes (L).

   (ii) **The Secretory Pathway,** given by Palade in 1960s, after carrying out the Pulse-chase experiments on the actively secreting pancreatic Acinar cells. He noticed that all the secretable proteins follow a common route to export, i.e. Translation by ER attached Ribosomes → RER lumen → TVs → cisGA → transGA → Discharge Vesicles → Cell Exterior. Later researches established that the same route is also taken by the proteins which are to be targeted to the PM, Lysosomes, and members of the Endomembrane system. This common pathway is called the *secretory pathway*.

   (iii) **Endomembrane Concept** is mainly a structural concept given by D. James Morre and Mollenhauer in 1974. The Endomembrane System consists of the endoplasmic reticulum, the nuclear envelope, and the Golgi apparatus. The system consists of internal compartments in

the cell which synthesize macromolecules and receive macromolecules from the outside environment.

Morphology

The ER in most of the cells appears as an anastomosing network of sacs (cisternae), tubules and vesicles enclosed by a continuous membrane which extends from the nuclear envelope (NE) throughout the cytoplasm. However, in certain cells the morphology of the ER can be different. The examples are as follows.

1. In striated muscle the ER is specially adapted to surround the myofibrils, forming triads with invaginations of the plasma membrane called T-tubules. This structure is called the *sarcoplasmic reticulum*.
2. In plants, Plasmodesmata are microscopic channels of protoplasm facilitating transport and communication between two adjacent cells. In the core of the plasmodesmata, there are *desmotubules*. The desmotubule is connected directly to the ER of each of the adjacent cells forming a channel between the ER of neighboring cells known as the dynamic endomembrane continuum.
3. In the fungi, belonging to Basidiomycota, the ER forms a special structure called *parenthosome*, around the dolipore septum that incompletely separates two adjacent cells.
4. The cells which are active in secretion, such as activated B-lymphocytes, have very well developed and highly branched ER system.

Rough and Smooth Endoplasmic Reticulum

In all cells, *there are two distinct types of ER* that appear different from one another and also perform different functions within the cell. However, *the two types of ER are in continuity with each other.*

1. The **Rough ER (RER)**, which is covered by ribosomes on its outer surface, functions in protein processing. Due to ribosome attachment, the RER has a granular surface, which becomes very apparent in scanning electron micrographs. The cisternal part of the ER is mostly rough type.
2. The **Smooth ER (SER)** is not associated with ribosomes, hence it has a smooth surface. It is involved in lipid metabolism. The smooth type of ER is more frequent on the margins of the cisternae, and also in the tubular and vesicular parts of the ER, as shown in Figure 2.

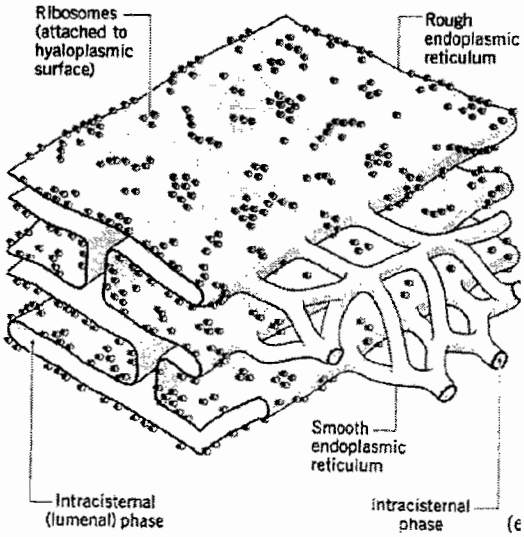


FIGURE 2: Rough and Smooth ER. Also shown, the lumen of the ER which is also called as Intra Cisternal Phase.

Structural Components

The ER has three structural components, namely:

1. The **Cisternae**, which are flattened vesicles having a large surface area. Their membrane is

about 9 nm thick and enclose a lumen (Intracisternal Phase) that is about 50-90 nm wide. The lumen of the ER is in continuity with the lumen of the nuclear envelope. The cisternae are frequently branched and inter-connected, and give the organelle the appearance of a reticulum.

2. The **Tubules**, which are elongated extensions from the margins of the cisternae. They have an elongated structure and their average diameter is about 30 nm. The tubules are mostly smooth. Like the cisternal part, even the tubules are frequently branched and interconnected.

3. The **Vesicles**, which surround the ER system near the margins. They are also mostly smooth. Their diameter varies from 80 nm to 120 nm.

Biochemical and Molecular Properties

To study the molecular organization and biochemistry of the ER, it is necessary to isolate the ER membrane. When tissues or cells are disrupted by homogenization, the ER breaks into fragments and reseals into many small (about 100–200 nm in diameter) closed vesicles called *microsomes*, which are relatively easy to purify.

Microsome membranes are equivalent to the ER membrane and the interior of the microsome is biochemically equivalent to the lumenal space of the ER.

The lumen of the ER has an oxidizing environment. It also contains several enzymes, for example the enzymes for protein glycosylation, lipid modification, cholesterol biosynthesis, xenobiotic modification etc. The CytP450 family of electron transfer proteins and Glucose-6-phosphatase are characteristically found in the ER membrane. Glucose-6-phosphatase is the histochemical marker enzyme for the ER.

The ER membrane, which has a lipid bilayer organization, is about 9 nm thick. It has several types of integral proteins. Some functionally important integral proteins of the ER membrane are CytP450, Ribophorins, Secretion (Sec) family of proteins, Signal Recognition Particle (SRP) Receptor, SNARE proteins, and Signal Peptidase.

It is these enzymes and proteins in the ER membrane and the ER lumen which make the ER functionally so versatile.

Functions

The Functions of the Smooth ER

1. In certain specialized cells such as the cells that specialize in lipid metabolism, the smooth ER may be abundant. This is because the SER membrane synthesizes nearly all of the major classes of lipids, including both phospholipids and cholesterol, required for the production of new cell membranes. The major phospholipid made is *phosphatidylcholine* (also called *lecithin*). The SER is the major site at which membrane lipids are synthesized in eukaryotic cells. As the lipids are extremely hydrophobic, they are synthesized in association with already existing cellular membranes rather than in the aqueous environment of the cytosol or in any aqueous lumen. Although some lipids are synthesized in association with other membranes, most are synthesized in the ER. They are then transported from the ER to their ultimate destinations either in vesicles or by carrier proteins.
2. In majority of cells, the SER sometimes acts as the *transitional ER* because it represents *ER exit sites* from which transport vesicles carrying newly synthesized proteins and lipids bud off for transport to the Golgi apparatus.

3. Cells that synthesize steroid hormones from cholesterol have an expanded SER compartment because many of the enzymes needed to make cholesterol and to modify it to form the hormones are located in the SER.
4. The main cell type in the liver, the *hepatocyte*, the SER is the principal site of production of lipoprotein particles, which carry lipids via the bloodstream to other parts of the body. The enzymes which synthesize the lipid components of lipoproteins are located in the membrane of the SER.
5. The SER also contains enzymes to detoxify both lipid-soluble drugs and various harmful compounds such as the drug phenobarbital. Mostly these *detoxification reactions* are carried out by the *cytochrome P450* family of enzymes, which catalyze the conversion of water-insoluble drugs or metabolites into water-soluble forms, which then leave the cell and be excreted in the urine.

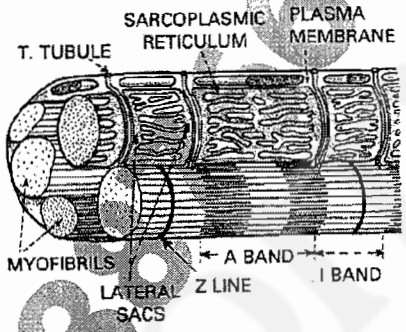


FIGURE 3: A part of the striated muscle, showing sarcoplasmic reticulum

6. The SER in most eukaryotic cells is to accumulate Ca^{2+} from the cytosol. The release of Ca^{2+} into the cytosol from the ER, and its subsequent reuptake, is involved in many rapid responses to extracellular signals. The storage of Ca^{2+} in the SER lumen is facilitated by the high concentrations of Ca^{2+}-binding proteins there.
7. The SER is also involved in the formation and storage of Glycogen in animal and fungal cells.
8. In the fungi, belonging to Basidiomycota, the ER forms a special structure called parenthosome, around the dolipore septum that incompletely separates two adjacent cells.
9. In striated muscle the ER is specially adapted to surround the myofibrils, forming triads with invaginations of the plasma membrane called T-tubules. This structure is called the *sarcoplasmic reticulum* (Figure 3). It accumulates Ca^{2+} from the cytosol by means of a Ca^{2+}-ATPase that pumps in Ca^{2+} into its lumen. The release and reuptake of Ca^{2+} by the sarcoplasmic reticulum trigger the contraction and relaxation, respectively, of the myofibrils during each round of muscle contraction.
10. In plants, the SER extends to give rise to a tubular structure called the *Desmotubule* (Figure 4), which runs through the plasmodesmata and acts as a connecting tubule between two adjacent cells. The desmotubule is associated with a specialized proteins to carry out a role in cell to cell cytoplasmic communication.
11. During the mitotic division of a plant cell, there is a special mechanism of cyto-kiensis which is different from animal cells. The animal cells undergo cytokinesis by the formation of a contractile ring. But, the plant cells form a *cell plate* during the telophase. In the formation of the cell plate, the fragments of SER cisternae along with the microtubules, contribute the first structural blocks, called the *phragmoplasts* (Figure 5).

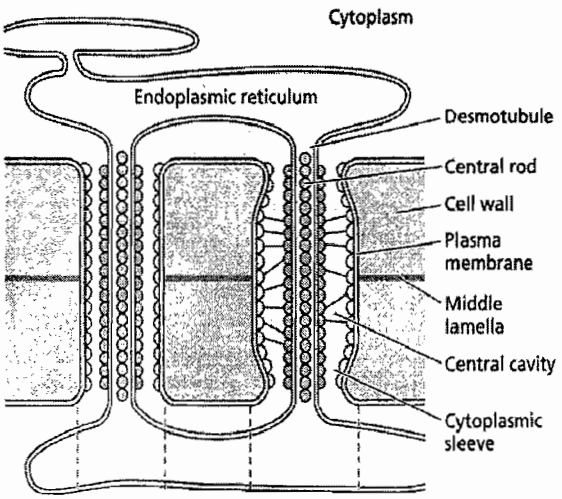


FIGURE 4: A section through a plant cell wall showing plasmodesmata and the desmotubule.
The desmotubules are extensions of SER and are associated with a number of structural proteins.

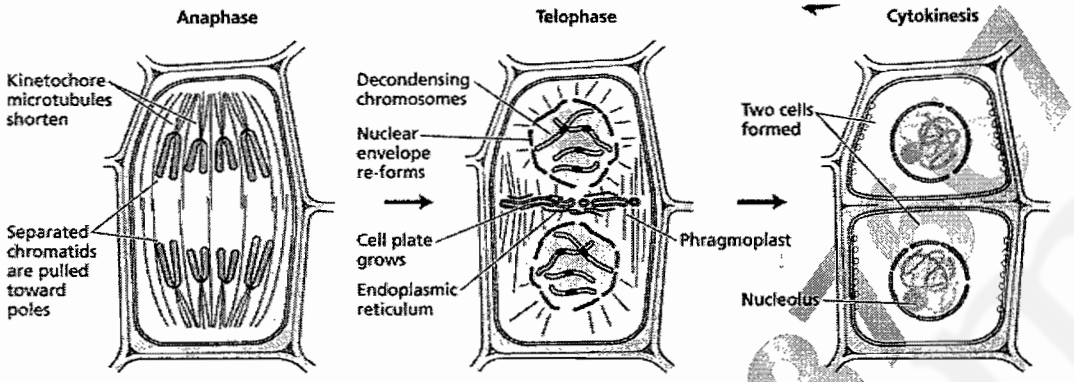


FIGURE 5: The late stages of plant cell division, showing the Cell Plate formation during the Telophase from SER derived phragmoplasts.

The Functions of the Rough ER

The term rough endoplasmic reticulum is based on the morphologic appearance of attached ribosomes, which are actively involved in translation.

The main function of the rough endoplasmic reticulum membrane is to serve as a site of entry of secretory, lysosomal, and integral plasma membrane proteins. These proteins undergo their initial chemical modification inside the lumen of the RER. The most important initial chemical modifications include attachment of oligosaccharides (glycosylation).

The process of entry of the secretory, lysosomal, and integral plasma membrane proteins into the RER lumen is described by the *Signal Hypothesis* (Gunter Blobel and David Sabatini, 1972). The mechanism is described below.

1. An mRNA for a specific peptide comes from the nucleus into the cytosol for translation. Translation of this mRNA starts with free floating ribosomes in the cytosol. As about 15-35 amino acids are added, the N-terminal acts as a *signal sequence*.
2. The signal sequence of the protein is recognized by a signal recognition particle (SRP) *while the protein is still being synthesized on the ribosome.* The SRP is a ribonucleoprotein (made of cytoplasmic RNA and six proteins) and by its binding the protein synthesis pauses for a while.
3. Now, the ribosome-protein complex is transferred to an SRP receptor on the rough endoplasmic reticulum.
4. There, the nascent protein is inserted into the Sec61 translocation complex (also known as the *translocon*) that passes through the ER membrane. The SRP gets released by a mechanism involving GTP hydrolysis.
5. The signal sequence is immediately cleaved from the polypeptide once it has been translocated into the ER by signal peptidase in secretory proteins.
6. The nascent protein grows within the RER lumen.
7. Once translation is over, the ribosome is released and the translocon closes.
8. Within the RER lumen, the protein is first covered by a chaperone protein to protect it from the high concentration of other proteins in the ER.Within the RER lumen, the nascent protein is also modified as needed (especially, by N-linked glycosylation). Both these processes are important for the nascent protein to fold correctly.

   This partly modified protein is then transported to the Golgi apparatus for further processing and goes to its target organelles.

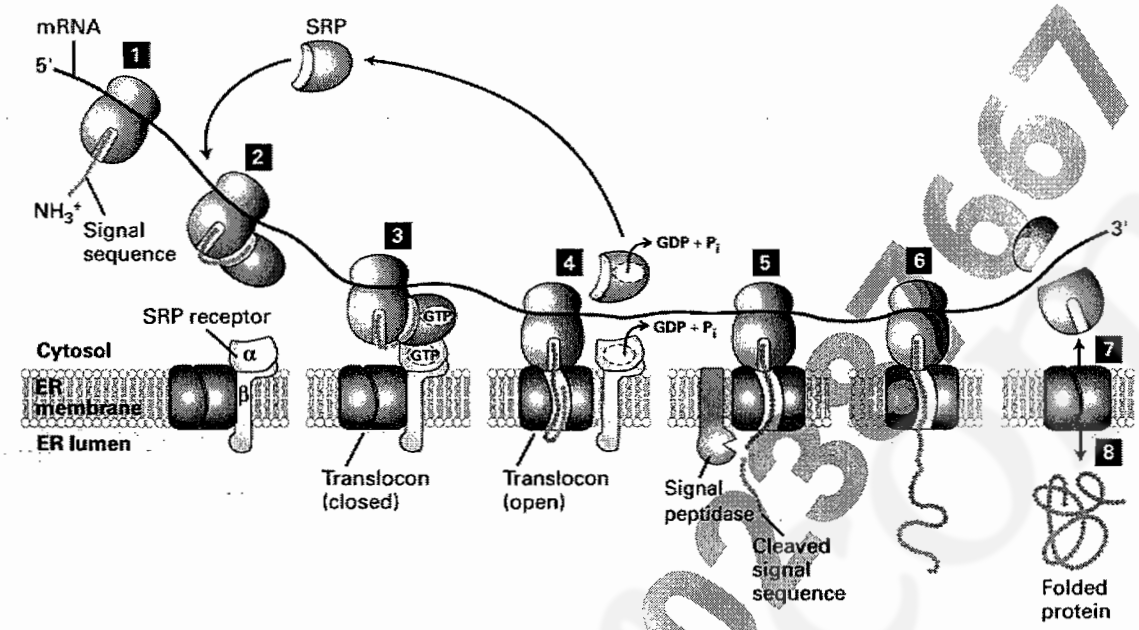


Figure 6: The steps involved in the entry of the secretory, lysosomal, and integral plasma membrane proteins into the RER lumen as described by the *Signal Hypothesis* (Gunter Blobel and David Sabatini, 1972). For the explanation of the numbered events, see the text above the figure.

Recently, in 2002 the scientists have also elaborated a new pathway of post-translational translocation of proteins into the RER lumen in eukaryotes. Many proteins in yeast (up to 60%), probably a few proteins in mammalian cells, are targeted to the ER after their translation is complete (post translational translocation), rather than being transferred into the ER during synthesis on membrane-bound ribosomes.

These proteins are synthesized on free cytosolic ribosomes, and their posttranslational incorporation into the ER *does not require SRP*. Instead, their signal sequences are recognized by distinct receptor proteins (the **Sec63 complex**) associated with the Sec61 complex in the ER membrane. Cytosolic chaperones, **hsc70** are required to maintain the polypeptide chains in an unfolded conformation so they can enter the Sec61 channel, and another chaperone within the ER lumen (called **BiP**) is required to pull the polypeptide chain through the channel and into the ER. The binding of polypeptide chains to BiP is a process dependent on ATP hydrolysis, which is facilitated by the Sec63 complex. The current understanding of this process is summarized in the following figure.

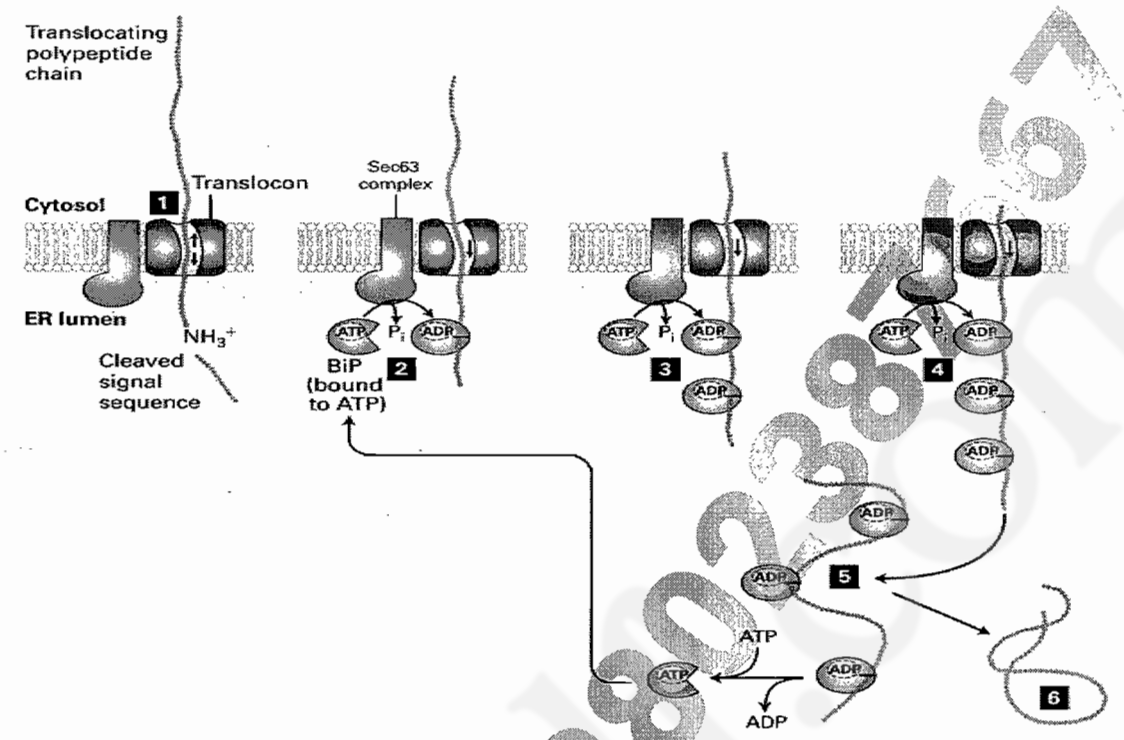


Figure 7: Post Translational entry of proteins into the RER lumen.

Protein Folding and Processing in the ER

Most plasma-membrane and secretory proteins contain one or more carbohydrate chains. The addition and subsequent processing of carbohydrates (*glycosylation*) is the principal chemical modification to most such proteins. Some glycosylation reactions occur in the lumen of the RER; others, in the lumina of the *medial* Golgi cisternae.

In glycoproteins, sugars are attached either to the amide nitrogen atom in the side chain of asparagine (termed an N-*linkage*) or to the oxygen atom in the side chain of serine or threonine (termed an O-*linkage*). N-linked glycosylation occurs in the RER lumen while the O-linked glycosylation occurs in the median Golgi lumen.

IN the ER, an asparagine residue can accept an oligosaccharide only if the residue is part of an **Asn-X-Ser** or **Asn-X-Thr** sequence, in which X can be any residue except proline.

A large oligosaccharide destined for N-linked attachment to the asparagine residue of a protein is assembled and attached to ***dolichol phosphate,*** a specialized lipid molecule in the RER membrane. Dolichol phosphate resides in the ER membrane with its phosphate terminus on the cytoplasmic face. It is towards the cytoplasmic side, where a part of the oligosaccharide is formed (upto the *pentascahharide* structure). Then, in a remarkable process, this large *pentascahharide* structure is "flipped" through the ER membrane into the lumen of the ER. This movement is facilitated by the class of enzymes known as **Flippases**. Finally, additional sugars are added to the *pentasaccharide core* by enzymes in the ER lumen. This process ends with the formation of a 14-residue oligosaccharide attached to dolichol phosphate.

The 14-sugar-oligosaccharide attached to this dolichol phosphate is transferred en-bloc to a specific asparagine residue of the growing polypeptide chain. This is a *one step enzymatic action catalysed by Glycosyl Transferase.*

There are four advantages of protein glycosylation.

1. Correct 3D folding is ensured for the protein
2. Stability of the proteins is enhanced
3. Cell-cell recognition is based on membrane glycoprotein's oligosaccharide parts.
4. Oligosaccharides aid in targeting too, although this does not appear to be the major role of glycosylation.

The RER is also the site of protein folding, assembly of multisubunit proteins, disulfide bond formation apart from the initial stages of glycosylation. The formation of disulfide bonds between the side chains of cysteine residues is an important aspect of protein folding and assembly within the ER. These bonds do not form in the cytosol, which is characterized by a reducing environment that maintains cysteine residues in their reduced (—SH) state. In the ER, however, an oxidizing environment promotes disulfide (S—S) bond formation. Disulfide bond formation is also facilitated by the enzyme protein disulfide isomerase [PDI], which is located in the ER lumen.

In addition, all the *alpha- helices of the protein are formed during its stay in the RER lumen itself.*

GOLGI APPARATUS

Introduction to Golgi apparatus

The **Golgi apparatus (GA)** (also called a **Golgi body**, **Golgi complex**, or **dictyosome**) is an organelle found in most eukaryotic cells (including those of plants, animals, and fungi) as a **part of the endomembrane system**. The GA is organised as a complex composed of flat sacs or cisternae stacked parallel in a protein matrix, called the **Golgi Matrix**. The location of the GA is between the ER and the Plasma Membrane.

The name comes from Italian anatomist Camillo Golgi, who identified it in 1898. The primary function of the Golgi apparatus is to process proteins targeted to the exoplasm, plasma membrane, lysosomes or endosomes. The Golgi apparatus is present in most eukaryotic cells, but tends to be more prominent where there are many substances, such as proteins, being secreted. For example, plasma B cells, the antibody-secreting cells of the immune system, have prominent Golgi complexes.

The Golgi Apparatus is the final processing and sorting centre for all those cellular proteins that are being targeted by the **secretory pathway** (Fig. 1). The GA processes these proteins and sort them within coated discharge vesicles. Most of the transport vesicles that leave the *rough* ER, are transported to the Golgi apparatus, where they are modified, sorted, and shipped towards their final destination. Thus, it functions as a protein delivery system for the cell.

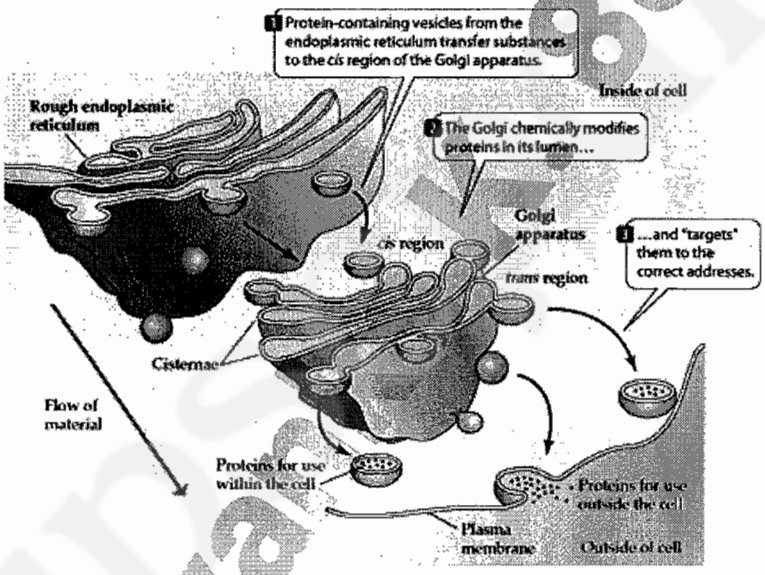


FIGURE 1: The position of the Golgi Apparatus in the overall secretory pathway.

Organization

A Golgi complex is composed of flat sacs or cisternae that are stacked parallel in a protein matrix, called the **Golgi Matrix**. The location of the GA is between the ER and the Plasma Membrane.

Each of GA usually consists of four to six cisternae although some unicellular flagellates can have up to 60. In animal cells, some stacks are linked by tubular connections between corresponding cisternae, thus forming a single complex, which is usually located near the cell nucleus and close to the centrosome. This localization depends on microtubules. If microtubules are experimentally depolymerized, the Golgi apparatus reorganizes into individual stacks that are found throughout the cytoplasm. In some cells, including most plant cells, hundreds of individual Golgi stacks are normally dispersed throughout the cytoplasm – where the GA is known as Dictyosome.

The Golgi complex is polar. The parallel-arranged cisternae are differentiated into 5 compartments (Fig 2):

(From ER towards the PM)

1. Cis Gogi Network
2. Cis Golgi Cisterna
3. Median Golgi Cisternae
4. Trans Golgi Cisterna
5. Trans Golgi Network

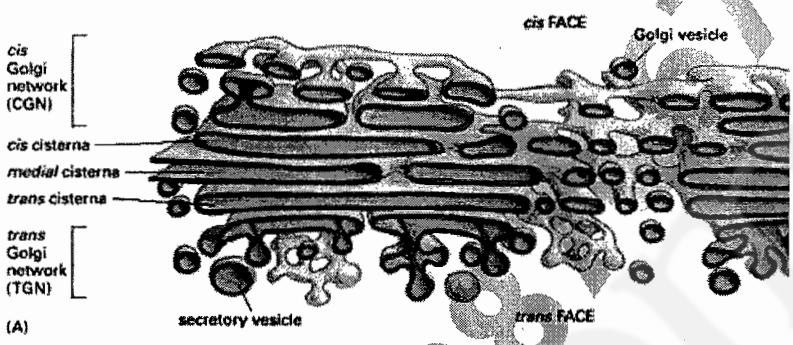


FIGURE 2: The various compartments of Golgi Apparatus

Each Golgi stack has two distinct faces: a ***cis* face** (or entry face) and a ***trans* face** (or exit face). Both *cis* and *trans* faces are closely associated with special compartments, each composed of a network of interconnected tubular and cisternal structures: the ***cis* Golgi network (CGN)** (also called the *intermediate compartment)* and the ***trans* Golgi network (TGN)**, respectively. Proteins and lipids enter the *cis* Golgi network in vesicular tubular clusters arriving from the ER and exit from the *trans* Golgi network bound for the cell surface or another compartment. Both networks are thought to be important for protein sorting.

There are **two possible models explaining the organization of the Golgi apparatus and the transport of proteins from one cisterna to the next**. It is likely that the transport through the Golgi apparatus in the forward direction involves elements of both of the views represented here.

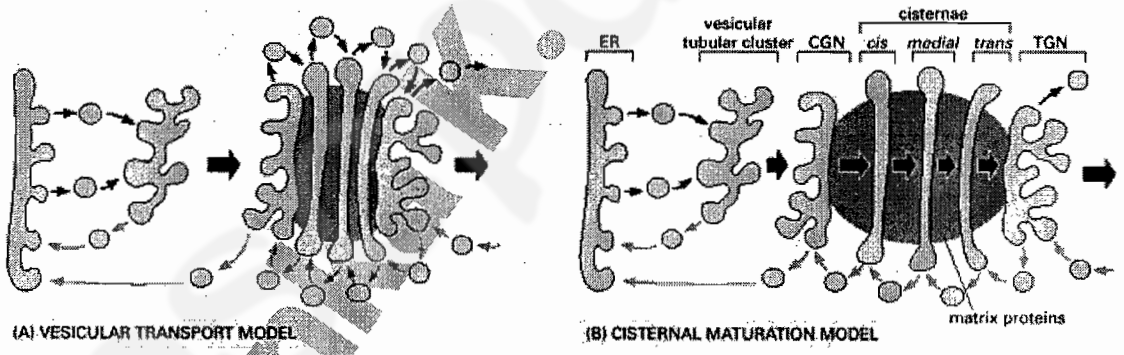


FIGURE 3: Two possible models explaining the organization of the Golgi apparatus and the transport of proteins from one cisterna to the next

1. In the **Vesicular Transport Model,** Golgi cisternae are static organelles, which contain a characteristic complement of resident enzymes. The passing of molecules through the Golgi is accomplished by forward-moving transport vesicles, which bud from one cisterna and fuse with the next in a *cis*-to-*trans* direction.

2. According to the **Cisternal Maturation Model,** each Golgi cisterna matures as it migrates outwards through a stack. At each stage, the Golgi resident proteins that are carried forward in a cisterna are moved backward to an earlier compartment in COPI-coated vesicles. When a newly formed cisterna moves around to a *medial* position, for example, "left-over" *cis* Golgi enzymes

would be extracted and transported backward to a new cis cisterna behind. Likewise, the *medial* enzymes would be received by retrograde (backwardly directed) transport from the cisternae just ahead. In this way, a cis cisterna would mature to a medial cisterna as it moves. Presently, the cell biologists largely favour the cisternal maturation model.

In **The Journal of Cell Biology**, Volume 155, Number 4, **November 12, 2001** (557-570) **Theresa H. Ward, Roman S. Polishchuk, Steve Caplan *et al*** reported that all classes of Golgi components are dynamically associated with this organelle, contrary to the earlier prediction of the stable organelle model. When ER to Golgi transport is inhibited without disrupting COPII-dependent ER export machinery, the Golgi structure disassembles, leaving no residual Golgi membranes. This observation also supports the cisternal maturation model.

A composite view of the dynamic assembly of the GA based on the Cisternal Maturation Model is shown in Figure 4.

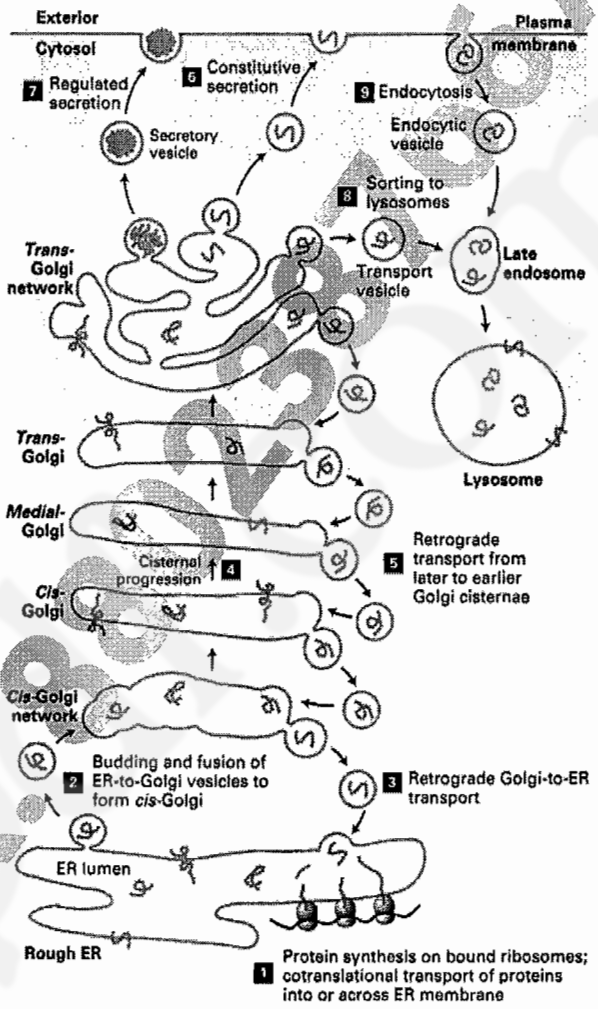


FIGURE 4: A composite view of the dynamic assembly of the GA based on the Cisternal Maturation Model

Functions

1. The Golgi apparatus is considered more or less the ***post office* of the cell**. It handles the targeting of proteins through the secretory pathway. The transport vesicles from the Endoplasmic Reticulum (ER) fuse with the *cis* face of the Golgi apparatus (to the cisternae) and empty their protein content into the Golgi lumen. The proteins are then transported through the medial region toward the *trans* face and are modified on their way. The modifications are shown in a diagram below.

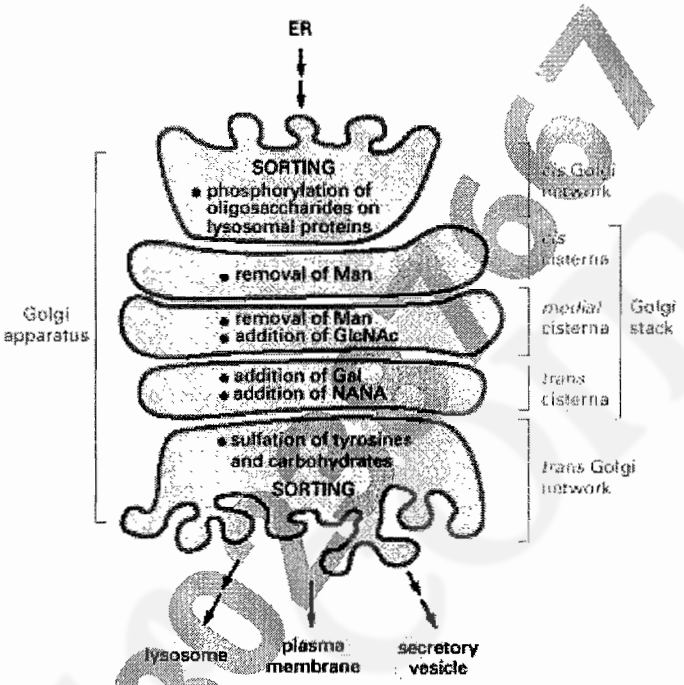
2. **The most important protein modification in the GA is O-linked glycosylation at specific Ser / Thr residues in the X-Ser/Thr-X context.** Vesicles from the ER contain simplified glycosylated proteins. ***O*-linked glycosylation** is catalyzed by a series of glycosyl transferase enzymes that use the sugar nucleotides in the lumen of the Golgi apparatus to add sugar residues to a protein one at a time. Usually, ***N*-acetylgalactosamine (GalNAc) is added first**, followed by a variable number of additional sugar residues (such as Glucose, Fucose, Sialic Acid and GlcNAc) ranging from just a few to 14 or more.

3. In the Golgi complex, N linked carbohydrates are edited from these semi-processed glycoproteins, creating a diversity of carbohydrate structures on the proteins.

4. The proteins are also labeled with a sequence of molecules according to their final destination. For example, the **Golgi apparatus adds a mannose-6-phosphate label to proteins destined for lysosomes**.

5. Further protein modifications like **Sulfonation, Adenylation** and **proteolytic cleavage of zymogens** also occur in the transGA.

6. GA also **sorts the proteins into appropriate discharge vesicle for their correct targeting**.



7. It is **a major site of carbohydrate synthesis,** as well as a sorting and dispatching station for the products of the ER. Many of the cell's polysaccharides are made in the Golgi apparatus, including the **pectin and hemicellulose of the cell wall in plants and most of the glycosaminoglycans of the extracellular matrix in animals**.

The GA Disassembly during Mitosis

The unique architecture of the Golgi apparatus depends on both the microtubule cytoskeleton and cytoplasmic Golgi matrix proteins, which form a scaffold between adjacent cisternae and give the Golgi stack its structural integrity. Some of the matrix proteins form long, filamentous tethers that are thought to help retain Golgi transport vesicles close to the organelle. When the cell prepares to divide, mitotic protein kinases phosphorylate the Golgi matrix proteins, causing the Golgi apparatus to fragment and disperse throughout the cytosol. During disassembly, Golgi enzymes are returned in vesicles to the ER, while other Golgi fragments are distributed to the two daughter cells. There, the matrix proteins are dephosphorylated, leading to the reassembly of the Golgi apparatus.

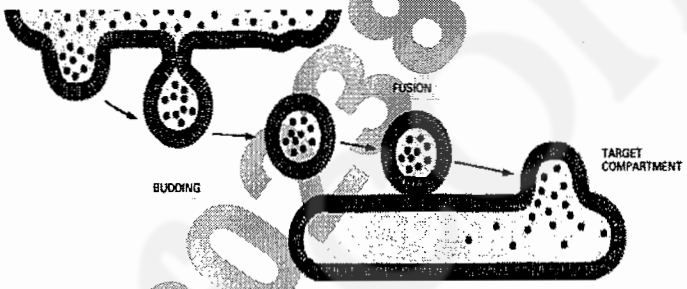
Remarkably, the Golgi matrix proteins can assemble into appropriately localized stacks near the centrosome even when Golgi membrane proteins are experimentally prevented from leaving the ER. This observation suggests that the matrix proteins are largely responsible for both the structure and location of the Golgi apparatus

VESICLE TRANSPORT

Introduction

Vesicular Transport (VT) is a eukaryotic method of sub-cellular trafficking of macromolecules (*especially proteins*) within transport vesicles.

FIGURE 7: THE BASIC DESIGN OF VESICULAR TRANSPORT IN A EUKARYOTIC CELL



A vesicle is a small single membrane enclosed entity in a eukaryotic cell's cytosol containing some substance in its lumen. The vesicles are generally of two types:

1. Structural vesicles, as we see in case of the Endoplasmic Reticulum organization
2. Transport vesicles, which are protein coated and serve to transport macromolecules from one location to another in a eukaryotic cell.

The cell organelle or the membrane that gives rise to the transport vesicles is called the **donor** and the cell organelle or the membrane that receives these vesicles is called the **target**. The substance carried by the transport vesicles is referred to as **cargo**. Transport vesicles bud off continually from one membrane and fuse with another, carrying membrane components and cargo. As they do so, they carry material from the donor compartment to the target compartment, as shown in Fig. 1.

The role played by VT

The transport vesicles are enclosed compartments for high efficiency macromolecular trafficking in a eukaryotic cell. A given transport vesicle will always fuse with a correct target membrane, thereby maintaining a very high degree of transport precision. Moreover, the vesicles carry much larger substances, which can otherwise not cross a biological membrane on their own. And finally, the vesicles also provide a protected luminal environment to the cargo substances against the various cytosolic proteases etc.

VT is the transport method by which:

1. materials are endocytosized by the cells from the exoplasm
2. materials are exocytosized or secreted out of the cell
3. semi-processed proteins are trafficked from the Rough ER to cis-Golgi in the secretory pathway
4. fully-processed proteins are trafficked from the Trans-Golgi Network (TGN) to

Lysosomes in the secretory pathway
5. fully processed integral plasma membrane proteins are sent from the Trans-Golgi Network to the Plasma Membrane
6. semi-processed proteins are forwarded from one Golgi cisterna to next one
7. membrane components are returned from one Golgi cisterna to the previous one or to the ER
8. some biological agents are engulfed by the cell from the exoplasm in the regions of membrane caveolae.

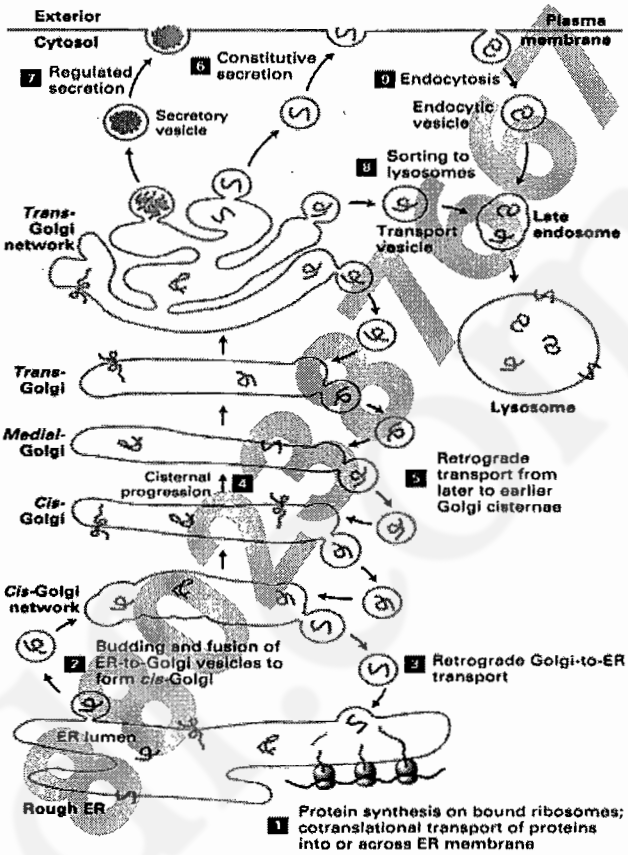


FIGURE 8: THE VARIOUS ORIGINS AND DESTINATIONS OF TRANSPORT VESICLES IN A EUKARYOTIC CELL

Types of Transport Vesicles based on Coat Proteins

All eukaryotic cells contain several types of transport vesicles, which all have a protein coat on their cytosolic surface at the time of mergence. **Five major types of coated vesicles are known**, each with a different type of protein coat. Each coat is formed by reversible polymerization of certain proteins. In the reversible polymerization of the coat proteins, a family of GTP binding proteins plays an important role.

Each type of vesicle transports proteins from particular parent organelles to particular destination organelles. For instance, vesicles with a **Clathrin coat** form from the plasma membrane and the *trans*-Golgi network (TGN) move to late endosomes. Vesicles with a **COP II (Coat protein II) coat** transport proteins from the export domain of the ER to the Golgi Apparatus via a transport intermediate, called vesicular tubular carrier (VTC). Vesicles with a **COP I (Coat protein I) coat** mainly transport proteins in the retrograde direction between Golgi cisternae and from the *cis*-Golgi back to the rough ER.

There are two newly discovered coat proteins too, in addition to the first three, which are known to the cell biologists for a long time. These two proteins are: **Radin**, coating the vesicles emerging from the TGN and fusing with the Plasma Membrane [Oddy et al, 2003] and **Caveolin**, which are nearly constitutively coated

upon the cytosolic face of the membrane **caveolae** and the vesicles emerging from them.

Fig. 3 shows how coat proteins and the destination of the vesicles are correlated.

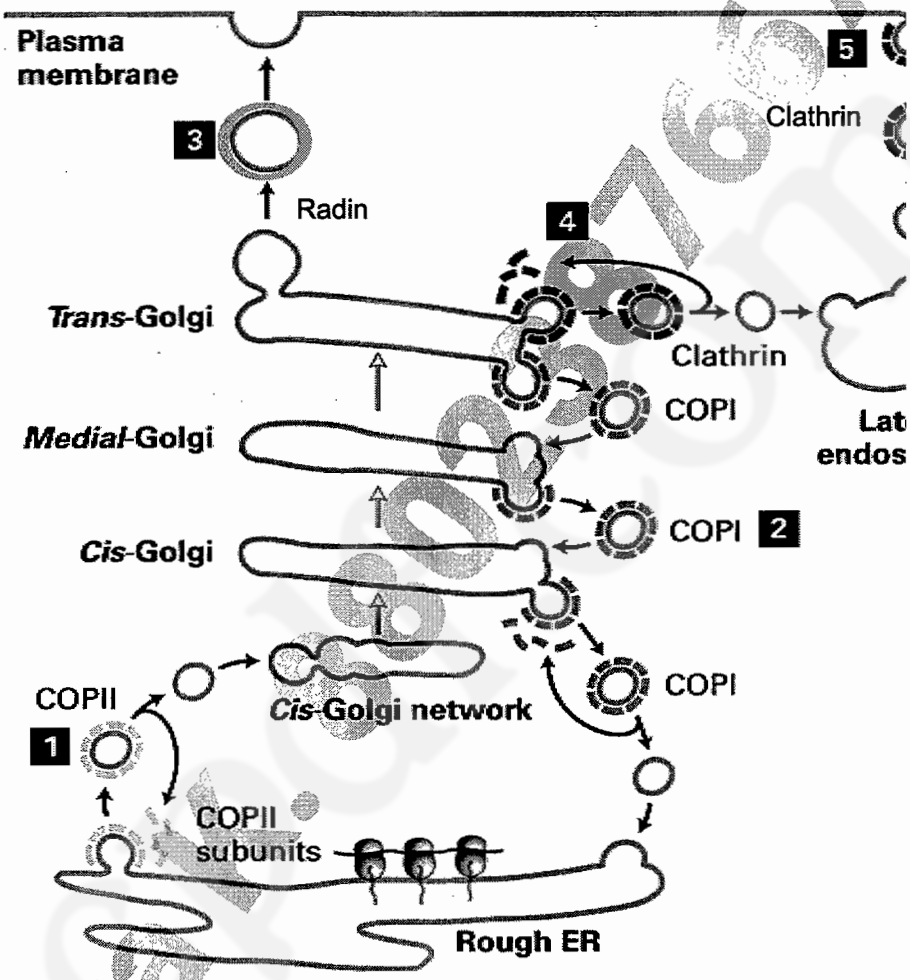
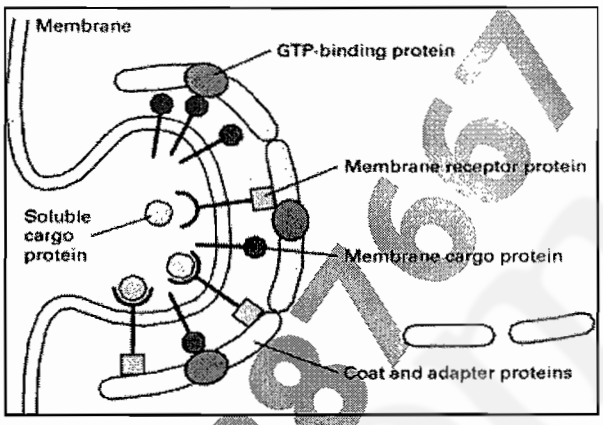


FIGURE 9: CORRELATION BETWEEN COAT PROTEINS AND THE DESTINATION OF THE VESICLES

The General Process of VT

The general scheme of vesicle budding and traffic applicable to all five known types of coated vesicles is as follows:

1. During formation of these vesicles, the coat subunit proteins polymerize around the cytosolic face of a budding vesicle, thereby helping the vesicle to pinch off from the parent organelle. In this polymerization, a GTP binding protein and an adapter protein play important roles. A feature common to formation of all types of protein coating around the vesicles is involvement of a GTP-binding protein that initiates coat polymerization.



2. Coat-protein subunits or associated adapter proteins [as in case of Clathrin coated vesicles] select which part of the donor membrane and soluble proteins will enter the transport vesicles as cargo proteins.
3. The cargo proteins can be of three types:
   a. Simple membrane bound
   b. Membrane bound receptors
   c. Soluble
4. These cargo proteins contain short signal sequences that direct them to a specific type of transport vesicle.
5. The coat proteins are depolymerised from the vesicle surface soon after the vesicle has successfully pinched off from the parent membrane and under transit towards the target membrane. In depolymerisation of the coat protein, the GTP binding protein hydrolises its bound GTP into GDP.
6. Depolymerisation of the coat proteins exposes the SNAREs, the membrane embedded proteins, which initiate membrane fusion between the vesicle and the target.
7. There are two types of SNAREs, 1. **v-SNAREs** [or the vesicular SNAREs] and 2. **t-SNAREs** [or the target SNAREs]. The SNARE proteins bind tightly with each other and initiate the fusion of the vesicular membrane with the target membrane.
8. Several other proteins are required to bring about vesicle fusion and vSNARE recycling.

Importance of vesicle transport

Transport vesicles play a central role in the traffic of molecules between different membrane-enclosed compartments of the secretory pathway. Vesicles are also involved in the transport of materials taken up at the cell surface. Vesicular transport is thus a major cellular activity, responsible for molecular traffic between a variety of specific membrane-enclosed compartments. The selectivity of such transport is key to maintaining the functional organization of the cell. For example, lysosomal enzymes must be transported specifically from the Golgi apparatus to lysosomes—not to the plasma membrane or to the ER.These proteins are transported within vesicles, so the specificity of transport is based on the selective packaging of the intended cargo into vesicles that recognize and fuse only with the appropriate target membrane. Because of the central importance of vesicular transport to the organization of eukaryotic cells, understanding the molecular mechanisms that control vesicle packaging, budding, and fusion is a major area of research in cell biology.

RIBOSOMES

Introduction to Ribosomes

The ribosomes are macro-molecular complexes of RNA and proteins – involved in Peptide Synthesis – found abundantly in the cell cytoplasm of both prokaryotes and eukaryotes. On an average, a bacterial cell contains about 25,000 ribosomes, which make about 25% of the total dry cellular mass. A eukaryotic cell has between 1–10 million ribosomes in its cytoplasm.

Chemically ribosomes are:

~ 75% RNA, known as ribosomal RNA (rRNA)

~ 25% proteins.

The role of the ribosome is to hold the complex of mRNA and tRNAs together during the translation stage of peptide synthesis, catalyze the peptide bonds formation between the amino acids and ensure the accuracy of protein synthesis.

Occurrence and Types

1. In prokaryotes and Archaea: 70S type. Always free floating in the cytoplasm mostly aggregated in the nucleoid region.
2. In eukaryotes: 80S type with three possible locations.
    a. Free floating in the cytoplasm.
    b. Attached to the RER membrane. In fact, it is the association of the ribosomes to the ER membrane surface because of which a particular region of the ER comes to be designated as RER.
    c. In eukaryotic nucleus: The 80S type of Ribosomes are also found in small quantities (~2%) in the eukaryotic nuclei – for the purpose of *mRNA Surveillance*. mRNA surveillance is a nuclear process for the detection and destruction of mRNAs that contain premature termination codons by a process called nonsense-mediated decay. (M.R. Culbertson, 2003).
3. In eukaryotic organelles: Various types of ribosomes have been reported from the genetically semiautonomous eukaryotic organelles viz. mitochondria (Mt) and the chloroplasts (Cp). Most of the plastidial ribosomes are of 70S type and they always occur in the chloroplast stroma. Mitochondrial ribosomes show great diversity. For example, among fungi, the Mt ribosome is 77S type, the Mt ribosome in mammals is of 55S type and in insects, the Mt ribosome is of 60S type. Mt ribosome is always found in the Mt matrix.

Ribosomal Architecture

The sub-units

Ribosomes consist of two subunits, large and small, that fit together. Prokaryotes have 70S ribosomes, each consisting of a (small) 30S and a (large) 50S subunit, whereas eukaryotes have 80S ribosomes, each consisting of a (small) 40S and a bound (large) 60S subunit.

The size of ribosomes and the RNA molecules within them have traditionally been measured by their sedimentation coefficient in **Svedberg Units, denoted by S**. This is the rate at which the particles sediment in a gradient, under centrifugal force, and takes into account the size, the shape, and density of the particle.

The ribosome's RNA and protein composition is summarized in the following diagram.

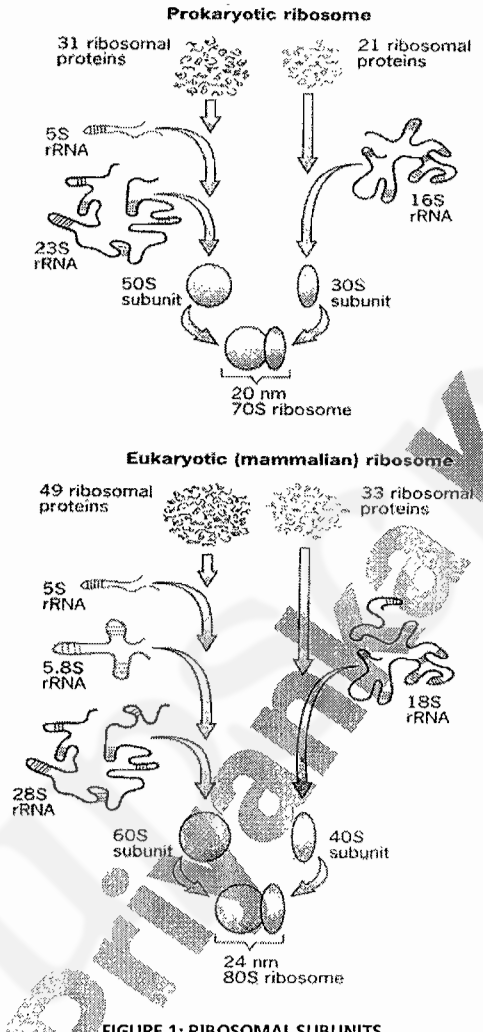


FIGURE 1: RIBOSOMAL SUBUNITS

Although ribosomes from prokaryotes and eukaryotes look similar in structure there are differences in the subunits and the composition of the proteins and rRNA within them. This makes protein synthesis an ideal target for antibiotics as drugs that affect bacterial ribosomes will not affect eukaryotic cells.

Molecular Anatomy and the Functional Domains of Ribosomes

Recently, four workers have separately unraveled the atomic structure of the ribosome. They are J. Frank (2000), N.Ban (2000), V.Ramakrishnan (2002) and P.B. Moore (2002). An EM structure was recently published by Mitra *et al.* (Nature, 2005).

V. Ramakrishnan is a scientist of Indian origin from the United States of America. He won the 2009 Nobel Prize for Chemistry for his pioneering work on the ribosomes. This breakthrough has led to a significant advancement in our current understanding about the structure and functions of the ribosomes.

Here we summarize our current knowledge about the ribosome's molecular anatomy and functional domains.

The rRNAs present in the ribosomes are crucial for both structure and function. Proteins only play a minor role in stabilizing the structure of the ribosomes.

1. The rRNAs are folded into well-defined conserved structures with many short duplex regions. Proteins interact

with the RNA mostly at the surface level.

2. The major functional domains in the ribosome are as follows.

   a. ***The mRNA binding site*:** It is located in the smaller subunit's solitary rRNA, i.e. 16S in prokaryotes and 18S in eukaryotes.

   b. ***The A (aminoacyl) site*:** The larger part of this site is located in the larger subunit but a smaller part of it extends into the smaller subunit also. This is the site, where during peptide chain elongation a new charged tRNA arrives and binds the codon.

   c. ***The P (peptidyl) site*:** Like the A site, this site is also mainly located in the large subunit but a part of it extends into the smaller subunit also. This is where a charged tRNA is placed when the peptide bond has been formed between the newly arrived amino acid and the last amino acid of the pre-existing peptide chain. The tRNA is moved to this site by ribosomal translocation.

   d. ***The E (exit) site*:** This is the site where a tRNA transiently binds the ribosome, before it leaves the complex after giving its amino acid. This site is almost entirely (~90%) located in the larger subunit of the ribosome.

   e. ***The Peptidyl transferase centre*:** This is the catalytic domain of the ribosome. It is entirely located in the larger subunit. In the prokaryotes, this catalytic centre is a part of the 23S rRNA. In eukaryotes, the catalytic centre is made of 28S rRNA. Its 3-D structure brings the amino acids together and catalyzes the formation of a peptide bond between them. For the presence of this catalytic centre, ribosomes are regarded as a ribozyme.

   f. ***The Peptide Exit tunnel*:** This is a tunnel composed of such rRNA domains, which cannot form any association with the nascent peptide chain. This tunnel occurs just above the P site and the newly growing peptide chain grows through this tunnel.

   g. ***A factor binding centre*:** It occurs in the large sub-unit, near the A site. Some translation factors which bind to GTP interact with this domain. The factor binding centre helps these translation factors to hydrolyze their bound GTP into GDP.

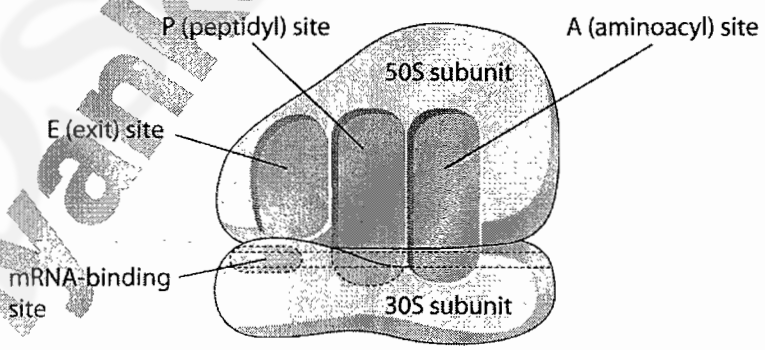


FIGURE 2: SOME FUNCTIONAL DOMAINS OF RIBOSOMES

Gross Morphology of Ribosomes

The currently accepted model of the ribosome morphology has been given by **James A. Lake** in 1981. Though initially given for the prokaryotic ribosome, this model is equally applicable to the eukaryotic ribosomes. It is summarized in Figure 3.

This completely asymmetrical model of ribosome suggested by James A. Lake (1981) essentially suggests the following.

1. The smaller subunit has
    a. A head
    b. A base
    c. A platform
2. The platform separates the head from the base by the help of a cleft. This cleft is an important functional region. It is suggested to be the site of codon-anticode interaction and as a part of binding site for initiation factors of protein synthesis.
3. The large subunit consists of
    a. A ridge
    b. A central protuberance
    c. A stalk
4. The ridge and the central protuberance are separated with the help of a valley.

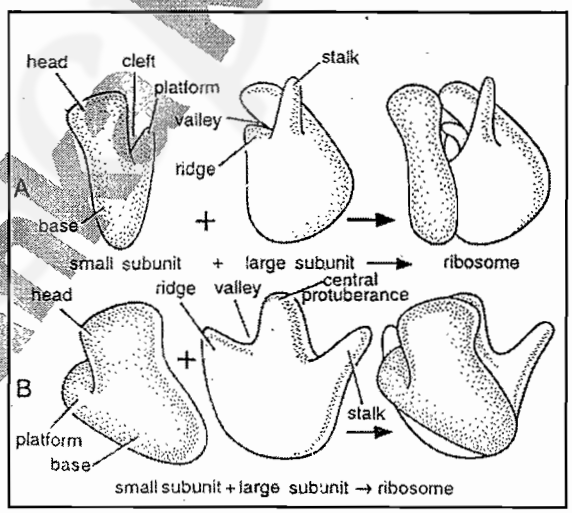


FIGURE 3: GROSS MORPHOLOGY OF RIBOSOMES

Functions & Significance

1. **Role of ribosome in cellular metabolism:** With its precise structure and catalytic activity, a ribosome acts as an assembly line for protein synthesis in the cytoplasm. This is one of the final steps of the *central dogma*. The central dogma says that DNA is used to make RNA, which, in turn, is used to make protein. The DNA sequence in genes is copied into a messenger RNA (mRNA). Ribosomes then read the information in mRNA and use it to create proteins. This process is known as translation because ribosome "translates" the genetic information from RNA into proteins. Ribosomes do this by binding to an mRNA and using it as a template for the correct sequence of amino acids in a particular protein. The amino acids are attached to transfer RNA (tRNA) molecules, which enter one part of the ribosome and bind to the messenger RNA sequence. The attached amino acids are then joined together by another part of the ribosome. The ribosome moves along the mRNA, reading its sequence and producing a chain of amino acids (Fig 3).

   By playing the fundamental role in protein synthesis, ribosomes have a central role in cellular metabolism. Proteins are essential parts of all organisms and participate in virtually every process within cells. Therefore, they have been called *biomolecules of first importance* (Gr. *Proteus* = First).

   Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that maintains cell shape. Other proteins are important in cell signaling, immune responses, cell adhesion, and the cell cycle.

   Thus, ribosomes provide for one of the most fundamental synthesis processes in a cell.

2. **Ribosomal RNA characteristics are important in medicine and in evolution**, as shown through the examples below.

   a. rRNA is the target of several clinically relevant antibiotics: Chloramphenicol, Erythromycin, Kasugamycin, Micrococcin, Paromomycin, Ricin, Sarcin, Spectinomycin, Streptomycin, and Thiostrepton.

   b. 16S / 18S rRNA is the most conserved (least variable) RNA in all cells. For this reason, genes that encode the rRNA (rDNA) are sequenced to identify an organism's taxonomic group, calculate related groups, and estimate rates of species divergence.

3. **Ribosomes have a role in *mRNA Surveillance* and *Non-sense Mediated Decay Pathways*.** These pathways are nuclear processes for the detection and destruction of mRNAs that contain premature termination codons. They were discovered and described by M.R. Culbertson in 2003.

CYTOSKELETON

Introduction

The cytosol is organized into *a three-dimensional network of filamentous proteins called the* ***cytoskeleton.*** This network serves three purposes:

1. It provides the spatial organization for the organelles;
2. It serves as scaffolding for the movements of organelles and other cytoskeletal components; and
3. It also plays fundamental roles in mitosis, meiosis, cytokinesis, wall deposition, the maintenance of cell shape, and cell differentiation.

Plant cells contain three types of cytoskeletal elements. They are:

1. Microtubules;
2. Microfilaments; and
3. Intermediate filament–like structures.

Each type is filamentous, having a fixed diameter and a variable length, up to many micrometers.

Microtubules

Microtubules are macromolecular assemblies of globular proteins.

Microtubules are hollow cylinders with an outer diameter of 25 nm; they are composed of polymers of the protein *tubulin*. The tubulin monomer of microtubules is a heterodimer composed of two similar polypeptide chains (α- and β-tubulin), each having an apparent molecular mass of 55,000 daltons (Figure 1). A single microtubule consists of hundreds of thousands of tubulin monomers arranged in *13 columns called protofilaments.*

In the cell, tubulin monomers exist as pools of free proteins that are in dynamic equilibrium with the polymerized forms. Polymerization requires energy: GTP (guano-sine triphosphate) for microtubule polymerization. The attachments between subunits in the polymer are noncovalent, but they are strong enough to render the structure stable under cellular conditions.

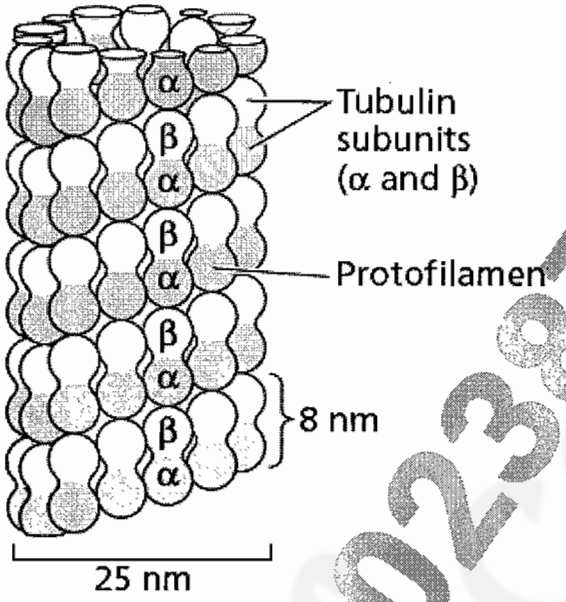


FIGURE 10: MOLECULAR STRUCTURE OF MICROTUBULE

Microtubules are polarized; that is, the two ends are different. In microtubules, the polarity arises from the polarity of the α- and β-tubulin heterodimer. The opposite ends of microtubules are termed plus and minus, and polymerization is more rapid at the positive end. Once formed, microtubules can disassemble. The overall rate of assembly and disassembly of these structures is affected by the relative concentrations of free or assembled subunits. In general, microtubules are more unstable. In animal cells, the half-life of an individual microtubule is about 10 minutes. Thus microtubules are said to exist in a state of *dynamic instability*.

Functions of microtubules

1. Microtubules function in mitosis and cytokinesis. Mitosis is the process by which previously replicated chromosomes are aligned, separated, and distributed in an orderly fashion to daughter cells. Micro-tubules are an integral part of mitosis. Before mitosis begins, microtubules in the cortical (outer) cytoplasm depolymerize, breaking down into their constituent subunits. The subunits then repolymerize before the start of prophase to form the *preprophase band* (PPB), a ring of microtubules encircling the nucleus. The PPB appears in the region where the future cell wall will form after the completion of mitosis, and it is thought to be involved in regulating the plane of cell division.

2. During prophase, microtubules begin to assemble at two foci on opposite sides of the nucleus, forming the prophase spindle. Although not associated with any specific structure, these foci serve the same function as animal cell centrosomes in organizing and assembling microtubules.

3. In early metaphase the nuclear envelope breaks down, the PPB disassembles, and new microtubules polymerize to form the mitotic spindle.

4. Cytokinesis is the process whereby a cell is partitioned into two progeny cells. Cytokinesis usually begins late in mitosis. The precursor of the new wall, the cell plate that forms between

incipient daughter cells, is rich in pectins. Cell plate formation in higher plants is a multistep process. Vesicle aggregation in the spindle midzone is organized by the phragmoplast, a complex of microtubules and ER.

5. Movements of organelles and other cytoskeletal components along the surfaces of microtubules occurs with help of motor proteins the kinesins and dyneins. This is important for proper localization of cellular components and vesicles.

Microfilaments

Microfilaments are macromolecular assemblies of globular proteins. Microfilaments are solid, with a diameter of 7 nm; they are composed of a special form of the protein found in muscle: globular actin, or *G-actin*. Each actin molecule is composed of a single polypeptide with a molecular mass of approximately 42,000 daltons. A microfilament consists of two chains of polymerized actin subunits that intertwine in a helical fashion (Figure 2).

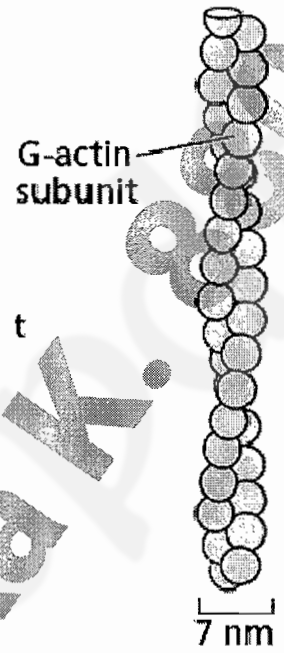


Figure 11: Structure of Microfilament

In the cell, actin monomers exist as pools of free proteins that are in dynamic equilibrium with the polymerized forms. Polymerization requires energy: ATP is required for microfilament polymerization. The attachments between subunits in the polymer are noncovalent, but they are strong enough to render the structure stable under cellular conditions.

Microfilaments are polarized; that is, the two ends are different. In microfilaments, the polarity arises from the polarity of the actin monomer itself. The opposite ends of microfilaments are termed plus and minus, and polymerization is more rapid at the positive end. Once formed, microfilaments can disassemble. The overall rate of assembly and disassembly of these structures is affected by the relative concentrations of free or assembled subunits. In general, microfilaments are more stable than microtubules.

Functions of Microfilaments

Microfilaments are involved in cytoplasmic streaming and in tip growth.

1. Cytoplasmic streaming is the coordinated movement of particles and organelles through the cytosol in a helical path down one side of a cell and up the other side. Cytoplasmic streaming occurs in most plant cells and has been studied extensively in the giant cells of the green algae *Chara* and *Nitella*, in which speeds up to 75 $\mu m\ s^{-1}$ have been measured.

   The mechanism of cytoplasmic streaming involves bundles of microfilaments that are arranged parallel to the longitudinal direction of particle movement. The forces necessary for movement may be generated by an interaction of the microfilament protein actin with the protein *myosin*. Myosins are proteins that have the ability to hydrolyze ATP to ADP and P*i* when activated by binding to an actin microfilament. The energy released by ATP hydrolysis propels myosin molecules along the actin microfilament from the minus end to the plus end. Thus, myosins belong to the general class of motor proteins that drive cytoplasmic streaming and the movements of organelles within the cell.

2. Actin microfilaments also participate in the growth of the pollen tube. Upon germination, a pollen grain forms a tubular extension that grows down the style toward the embryo sac. As the tip of the pollen tube extends, new cell wall material is continually deposited to maintain the integrity of the wall. A network of microfilaments appears to guide vesicles containing wall precursors from their site of formation in the Golgi through the cytosol to the site of new wall formation at the tip. Fusion of these vesicles with the plasma membrane deposits wall precursors outside the cell, where they are assembled into wall material.

Intermediate Filaments

Intermediate filaments are a diverse group of helically wound fibrous elements, 10 nm – 20 nm in diameter. Intermediate filaments are composed of linear polypeptide monomers of various types. For example, in animal cells the nuclear lamins are composed of a specific polypeptide monomer, while the keratins, a type of intermediate filament found in the cytoplasm, are composed of a different polypeptide monomer.

In intermediate filaments, pairs of parallel monomers (i.e., aligned with their —NH_2 groups at the same ends) are helically wound around each other in a coiled coil. Two coiled-coil dimers then align in an antiparallel fashion (i.e., with their —NH_2 groups at opposite ends) to form a tetrameric unit. The tetrameric units then assemble into the final intermediate filament (Figure 3).

Although nuclear lamins appear to be present in plant cells, there is as yet no convincing evidence for plant keratin intermediate filaments in the cytosol. Because the plant cell wall serves as a kind of cellular exoskeleton, it removes the need for keratin-type intermediate filaments for structural support.

In contrast to microtubules and microfilaments, intermediate filaments lack polarity because of the antiparallel orientation of the dimers that make up the tetramers. In addition, intermediate filaments appear to be much more stable than either microtubules or microfilaments. Although very little is known about intermediate filament–like structures in plant cells, in animal cells nearly all of the intermediate-filament protein exists in the polymerized state.

Functions of Intermediate Filaments

Relatively little is known about plant intermediate filaments. Intermediate filament–like structures have been identified in the cytoplasm of plant cells (Yang et al. 1995), but these may not be based on keratin, as in animal cells, since as yet no plant keratin genes have been found. Nuclear lamins, intermediate filaments of another type that form a dense network on the inner surface of the nuclear membrane, have also been identified in plant cells (Frederick et al. 1992), and genes encoding lamin-like proteins are present in the *Arabidopsis* genome. Presumably, plant lamins perform functions similar to those in animal cells as a structural component of the nuclear envelope.

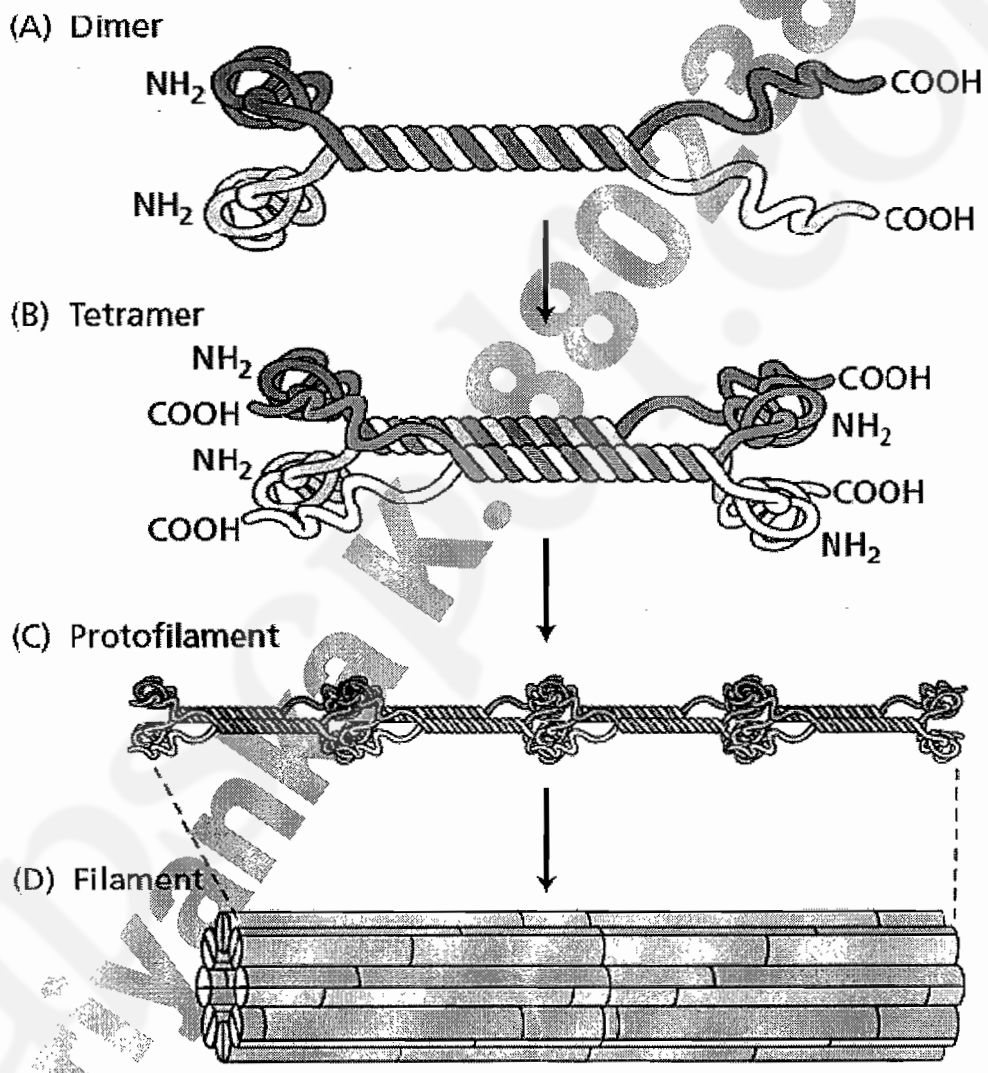


Figure 12: Organisation of Intermediate Filaments

LYSOSOMES

What are Lysosomes?

Lysosomes are single membrane-enclosed compartments in the eukaryotic cells filled with acid dependent hydrolytic enzymes that are used for the controlled intracellular digestion of macromolecules. They contain about 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. In essence, they contain an array of enzymes capable of breaking down all types of biological polymers—proteins, nucleic acids, carbohydrates, and lipids. Lysosomes function as the digestive system of the cell, serving both to degrade material taken up from outside the cell and to digest obsolete components of the cell itself. They digest excess or worn out organelles, food particles, and engulfed viruses or bacteria.

In their simplest form, lysosomes are visualized as dense spherical vacuoles, but they can display considerable variation in size and shape as a result of differences in the materials that have been taken up for digestion (*Refer to your Class Lecture Illustrations*). Lysosomes thus represent morphologically diverse organelles defined by the common function of degrading intracellular material.

Discovery & characterisation of lysosomes

The lysosomes were discovered and first characterized in 1949 by **Christian René de Duve**, an internationally acclaimed cytologist and biochemist.

In his studies, two types of small spherical bodies in the periphery of the cytoplasm were isolated by Christain De Duve. He characterized these isolated vesicles as **microbodies**, due to very small size.

Later, after, a biochemical analysis of these microbodies, they were categorized as:

1. Lysosomes: Which contain hydrolytic enzymes & can carry out lysis of cellular structures
2. Peroxisomes: Which can metabolize peroxide.

Early characterization of the lysosomes was as *suicide bags* of the cell, due to two **wrong** presumptions:

1. Lysosomes carry the enzymes capable of causing cell death
2. An accident burst of the lysosomes will kill the cell by degrading its components.

These presumptions are wrong, because:

Lysosomes play no significant role in cell death (apoptosis)

An accidental release of lysosomal enzyme will cause no harm to the cell. This is because all lysosomal hydrolytic enzymes show an optimal action only at acidic pH (below pH 5). Cytosolic pH is very moderately basic (~ pH 7.2), where the lysosomal enzymes would be ineffective. Even if lysosomes burst,

the matrix material released from the lysosomes would not be enough to acidify the cytosolic pH, at which the lysosomal hydrolytic enzymes would be active. Ultimately, no harm will be caused to the cell.

The Modern Concept of Lysosomes

Any single membrane bound eukaryotic organelle is a lysosome if:

1. It contains an acidic pH
2. It contains acid hydrolase enzymes (at least 5 different types of them)
3. Its enzymes show latency.

Following this concept, we can identify:

1. *Classical lysosomes* as recognized in animal cells
2. *Organelles regarded as lysosomes* which have traditionally been called by different names, but fulfill all the 3 criteria mentioned above.

   Examples include:

   a. Plant and fungal cell vacuoles (excluding the storage vacuoles)

   b. Secretory granules which release hydrolytic enzymes. Such gramules are wide spread among animals, fungi & Plants.

Lysosomal morphology

Lysosomes are morphologically heterogeneous (*Refer to your Class Lecture Illustrations*). The heterogeneity of lysosomal morphology contrasts with the relatively uniform structures of most other cell organelles. The diversity reflects the wide variety of digestive functions mediated by acid hydrolases, including the breakdown of intra- and extracellular debris, the destruction of phagocytosed microorganisms, and the production of nutrients for the cell. For this reason, lysosomes are sometimes viewed as a heterogeneous collection of distinct organelles whose common feature is a high content of hydrolytic enzymes. Overall, the lysosomal morphology depends on:

1. Source cell / organism from where lysosome has been isolated
2. Functional status of the lysosomes

The diameter of some representative lysosomal types is given below.

1. Classical animal lysosomes (primary state): 0.5 – 1 μm
2. Secondary Lysosomes of animal cells: 1 μm – 2 μm.
3. Animal secretory granules: 25—50 nm
4. Fungal secretory granules: 50 nm
5. Plant cell vacuole: ~ 10—15 μm

In terms of shape, lysosomes are mostly spherical & only sometimes they are oval, or irregularly shaped.

There are 3 morpho-functional classes of lysosomes (*Refer to your Class Lecture Illustrations*):

1. Primary lysosomes: A newly formed one, not yet active
2. Secondary lysosomes: A functional lysosome acting on its substrate
3. Residual body: A lysosome which contains undigested parts of substrate

Molecular structure & biochemistry

As already discusesd, lysosomes are single membrane-enclosed compartments filled with hydrolytic enzymes that are used for the controlled intracellular digestion of macromolecules. They contain about 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. Of them, any given lysosome contains between 15 and 25 enzymes.All lysosomal enzymes are are acid hydrolases. For optimal activity, they require an acid environment, and the lysosome provides this by maintaining a pH of about 5.0 in its interior.

The major classes of lysosomal enzymes & their representative members are tabulated below.

Table 1: A sampling of lysosomal enzymes

Enzyme	**Susbtrate**
Phosphatases	
Acid phosphatese	Phosphomonoesgters
Acid phosphodieserase	Phosphodiesters
Nucleases	
Acid ribonuclease	RNA
Acid deoxyribonuclease	DNA
Proteases	
Cathepsin	Proteins
Collagenase	Collagen
GaG-hydrolyzing enzymes	
Iduronate sulfatase	Dermatan sulfate
β-Galactosidase	Keratin sulfate
Heparan N-sulfatase	Heparan sulfate
α-N-Acetylglucosaminidase	Heparan sulfate
Polysaccharidases and Oligosaccharidases	
α -Glucosidase	Glycogen

Fucosidase	Fucosyloligosaccharides
α -Mannosidase	Mannosyloligosaccharides
Sialidase	Sialyloligosaccharides
Sphingolipid hydrolyzing enzymes	
Ceramidase	Ceramide
Glucocerebrosidase	Glucosylceramide
β -Hexosaminidase	G_{M2} ganglioside
Arylsulfatase A	Galactosylsulfatide
Lipid hydrolyzing enzymes	
Acid lipase	Triacylglycerols
Phospholipase	Phospholipids

Since for optimal activity all lysosomal enzymes require an acidic environment, the lysosome provides this by maintaining a pH of about 5.0 in its interior. An H^+ pump in the lysosomal membrane uses the energy of ATP hydrolysis to pump H^+ into the lysosome, thereby maintaining the lumen at its acidic pH. The proton pump in the lysosomal membrane requires expenditure of energy in the form of ATP hydrolysis, since it maintains approximately a hundredfold higher H^+ concentration inside the lysosome.

A similar or identical *vacuolar H^+ ATPase* (belonging to *V- class H^+ ATPase family*) acidifies all endocytic and exocytic organelles, including lysosomes, endosomes, selected compartments of the Golgi apparatus, and many transport and secretory vesicles.

Apart from a hydrogen ion pump, a Cl^- channel protein in the lysosomal membrane also contributes to maintain the pH of the interior at ≈4.8. The H^+ pump hydrolyzes ATP and uses the released free energy to pump H^+ ions from the cytosol into the lumen of the lysosome; while the Cl^- channel allows Cl^- ions to enter. Together they transport HCl. The acid pH helps to denature proteins, making them accessible to the action of the lysosomal hydrolases, which themselves are resistant to acid denaturation.

Most of the lysosomal membrane proteins are unusually highly glycosylated, which helps to protect them from the lysosomal proteases in the lumen.

The lysosome not only contains a unique collection of enzymes, but also has a unique surrounding membrane. Transport proteins in this membrane allow the final products of the digestion of macromolecules—such as amino acids, sugars, and nucleotides—to be transported to the cytosol, from where they can be either excreted or reutilized by the cell.

Lysosome formation & functional dynamics

Lysosome formation takes place in two phases.

1. **Formation of a primary lysosome**: A primary lysosome is nothing but a vesicle containing lysosomal enzymes. It pinches off from the Trans-Golgi Network. It contains lysosomal enzymes and lysososme specific *V- class H^+ ATPase* for proton pumping (which later acidifies the interior

of the lysosome). A primary lysosome is functionally not active, as it does not contain its substrate for the digestive enzymes to act upon.

The acid hydrolases are targeted to lysosomes by mannose-6-phosphate residues, which are recognized by mannose-6-phosphate receptors in the *trans* Golgi network and packaged into clathrin-coated vesicles. Following removal of the clathrin coat, these transport vesicles acts as primary lysosome.

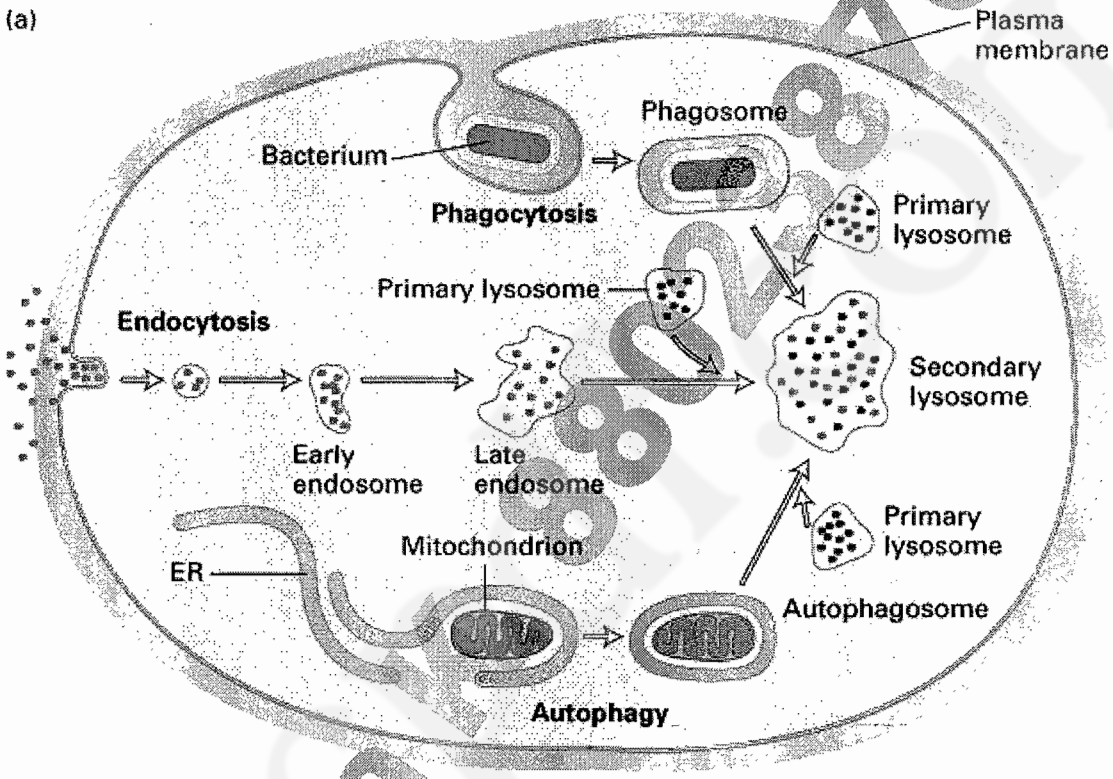


FIGURE 1: FORMATION OF AN ACTIVE LYSOSOME

2. **Formation of an active lysosome**: (See **Figure 1**) An active lysosome is formed when a primary lysosome fuses with a vesicle containing the substrate. This can happen under the following three circumstances.

   a. **Lysosome Formation & Function during Endocytosis**: One of the major functions of lysosomes is the digestion of material taken up from outside the cell by endocytosis. In such cases, active lysosomes are formed by the fusion of primary lysosome budded from the *trans* Golgi network with endosomes, which contain molecules taken up by endocytosis at the plasma membrane.Material from outside the cell is taken up in clathrin-coated endocytic vesicles, which bud from the plasma membrane and then fuse with early endosomes. Membrane components are then recycled to the plasma membrane and the early endosomes gradually mature into late endosomes, which are the precursors to active lysosomes. One of the important changes during endosome maturation is the lowering of the internal pH to about 5.5, which plays a key role in the activity of lysosomal acid hydrolases from the *trans* Golgi network.

The hydrolases when released into the lumen of the endosome, the late endosomes mature into active lysosomes as they acquire a full complement of acid hydrolases, which digest the molecules originally taken up by endocytosis.

b. **Lysosome Formation & Function during Phagocytosis**: In addition to degrading molecules taken up by endocytosis, lysosomes digest material derived from two other routes: phagocytosis too. In phagocytosis, specialized cells, such as macrophages, take up and degrade large particles, including bacteria, cell debris, and aged cells that need to be eliminated from the body. Such large particles are taken up in phagocytic vacuoles (**phagosomes**), which then fuse with primary lysosomes, resulting in digestion of their contents. The lysosomes formed in this way (**phagolysosomes**) can be quite large and heterogeneous, since their size and shape is determined by the content of material that is being digested.

c. **Lysosome Formation & Function during Autophagy**: Lysosomes are also responsible for autophagy, the gradual turnover of the cell's own components. The first step of autophagy appears to be the enclosure of an organelle (e.g., a mitochondrion) in membrane derived from the ER. The resulting vesicle (an **autophagosome**) then fuses with a primary lysosome, and its contents are digested. Autophagy is responsible for the gradual turnover of cytoplasmic organelles.

Apart from intra-cellular lysosomes, some lysosomes may also undergo exocytosis. ***Lysosomal secretion*** (also called *defecation*) of their undigested content enables all cells to eliminate indigestible debris. For most cells, this seems to be a minor pathway, used only when cells are stressed. Some cell types, however, contain specialized lysosomes that have acquired the necessary machinery for fusion with the plasma membrane. *Melanocytes* in the skin, for example, produce and store pigments in their lysosomes. These pigment-containing *melanosomes* release their pigment into the extracellular space by exocytosis. The pigment is then taken up by keratinocytes, leading to normal skin pigmentation. In some genetic disorders, this transfer process is blocked owing to defects in melanosome exocytosis, leading to forms of hypopigmentation (albinism).

Functional significance of lysosomes

Lysosomes are membrane-enclosed organelles that contain an array of enzymes capable of breaking down all types of biological polymers—proteins, nucleic acids, carbohydrates, and lipids. Lysosomes function as the digestive system of the cell, serving both to degrade material taken up from outside the cell and to digest obsolete components of the cell itself.

Certain important aspects of lysosomal functioning are given below.

Autophagy

Autophagy is the intracellular process by which the cell degrades its own components using the lysosomal machinery and recycles the molecules. Damaged macromolecules, malformed proteins, non-functional, long-lived proteins, and damaged and old organelles are all broken down by the lysosomal enzymes. A phospholipid membrane is formed around the target component, resulting in the formation of a vesicle called autophagic vacuole. This vacuole then fuses with the primary lysosome where the hydrolases digest the macromolecules to sugars, amino acids and nucleotides, which are the primary building blocks of every cell. These useful digestion products are released into the cytosol and can be utilized in the synthesis of

new macromolecules and organelles, whereas the unwanted products are released outside the cell. Also, during starvation, or nutrient-limiting conditions, autophagy of normal organelles occurs, thus helping to maintain the level of nutrients required for the normal cellular processes.

Role in Endocytosis and Phagocytosis

Endocytosis is the process for cellular uptake of foreign material. Phagocytosis is a specialized form of endocytosis wherein large bodies such as dead cells, cell debris, bacteria, viruses, etc., are engulfed. The uptake occurs through specialized vesicles which fuse with the lysosomes followed by the degradation of the foreign entity by hydrolases. Directly or indirectly, both these processes play a crucial role in pathogen destruction.

Role in Apoptosis

Apoptosis or programmed cell death is a very intricately controlled mechanism of cellular suicide. Such a mechanism is essential during embryonic development and for destruction of old cells, infected cells and cells with DNA damage. Apoptosis is initiated through various pathways in a cell. In late stages of apoptosis, lysosomes have been identified to clear cell debris.

After apoptosis, the components of the dead cell are packed into vesicles termed apoptotic bodies which are engulfed by neighboring cells. The lysosomes of the neighboring cells bring about the residual digestion of these components.

Role in Fertilization

Lysosomes are also capable of releasing their digestive enzymes outside the cell to bring about extracellular digestion. During fertilization, the lysosomal contents of sperms are released outside the cell in order to bring about the digestion of the limiting membrane around the egg. This facilitates fusion of the sperm and egg. In addition to this, once the two cells fuse, the paternal mitochondria are destroyed through the lysosomal machinery of the egg. Sperm-derived mitochondria tend to accumulate genetic mutations due to the high metabolic activity of sperms. Hence, they need to be eliminated from the fused cell to avoid the transfer of mutations to the resulting embryo.

Cell Membrane Repair

Conditions of mechanical stress and pathogenic actions can lead to disruption of certain patches or formation of pores in the cell membrane. The secretory lysosomes fuse with the cell membrane at a location close to the damaged patch. This results in the release of hydrolases outside the cell. Of these hydrolases, a specialized hydrolase called acid sphingomyelinase (ASM) causes the internalization of damaged patch by the cell. Moreover, the fusion of the lysosome with the membrane provides extra lipids and prevents constriction of the cellular boundary.

Diseases resulting from lysosomal malfunctioning

Mutations in the genes that encode these enzymes are responsible for more than 30 different human genetic diseases, which are called **lysosomal storage diseases** because undegraded material accumulates within the lysosomes of affected individuals. Most of these diseases result from deficiencies in single lysosomal enzymes. Some important examples are mentioned below.

1. *Gaucher's disease* results from a mutation in the gene that encodes a lysosomal enzyme required for the breakdown of glycolipids.

2. *Tay-Sachs disease* is caused by a defect in one enzyme catalyzing a step in the lysosomal breakdown of certain glycolipids called gangliosides, which are abundant in nerve cells—with devastating consequences. The symptoms of this inherited disease usually are evident before the age of 1. Affected children commonly become demented and blind by age 2, and die before their third birthday. Nerve cells from such children are greatly enlarged with swollen lipid-filled lysosomes.

3. In *Hurler's disease,* the enzyme required for the breakdown of glycosaminoglycans is defective or missing.

4. The most severe form of lysosomal storage disease, however, is a very rare disorder called *Inclusion-cell disease* (*I-cell disease*). In this disease, almost all of the hydrolytic enzymes are missing from the lysosomes of fibroblasts, and their undigested substrates accumulate in lysosomes, which consequently form large "inclusions" in the patients' cells. I-cell disease is caused by a deficiency in the enzyme that catalyzes the first step in the tagging of lysosomal enzymes with mannose-6-phosphate in the Golgi apparatus. The result is a general failure of lysosomal enzymes to be incorporated into lysosomes.

ENDOSOMES

Introduction to endosomes

Endosomes are large vesicles which are formed from the plasma membrane derived primary endocytic / phagocytic vesicles as a result of endocytosis / phagocytosis.

There are four types of vesicles which lead to the formation of endosomes.

1. Vesicles arising from receptor mediated endocytosis. Such vesicles are clathrin coated.
2. Vesicles arising from caveolae on the plasma membrane. They are coated with a protein called caveolin.
3. Vesicles arising from phagocytosis. They are uncoated. Phagocytosis ("cellular eating") involves the ingestion of large particles, such as microorganisms or cell debris, via large vesicles called *phagosomes*, generally > 250 nm in diameter.
4. Vesicles arising from pinocytosis. They are uncoated. Pinocytosis ("cellular drinking") involves the ingestion of fluid and solutes via small vesicles (≤ 150 nm in diameter).

The endosomes are intermediate compartments of the endocytic membrane transport pathway from the plasma membrane to the lysosome. Molecules internalized from the plasma membrane follow this pathway to lysosomes for degradation.

Formation of endosomes

Endosomes arise from endocytosis derived vesicles. Material to be ingested is progressively enclosed by a small portion of the plasma membrane, which first invaginates and then pinches off to form an intracellular vesicle containing the ingested substance or particle. The vesicle thus formed is called a primary endocytic vesicles. These vesicles fuse to give rise to endosomes. Endosomes are approximately 500 nm in diameter when fully mature.

Phases of endosomes

As shown in *Figure 1*, Endosomes comprise three different compartments: *early endosomes or EEs*, *late endosomes or LEs*, and *recycling endosomes or REs*.

The primary endocytic vesicles deliver their contents and their membrane to EEs in the peripheral cytoplasm.

For a period of about 8–15 min during which the EEs accumulate cargo and support recycling to the plasma membrane (directly or via REs in the perinuclear region).

After this conversion of the EEs to LE takes place. As the endosomes are moving towards the perinuclear space along microtubules (MT), the nascent LE are formed. They carry a selected subset of endocytosed cargo from the EE. The LEs combine *en route* with newly synthesized lysosomes.

The fusion of an endosome with a lysosome generates a transient hybrid organelle, the endolysosome, in which active degradation takes place.

Later, the endolysosome is converted to a classical dense lysosome, which constitutes a storage organelle for lysosomal hydrolases and membrane components.

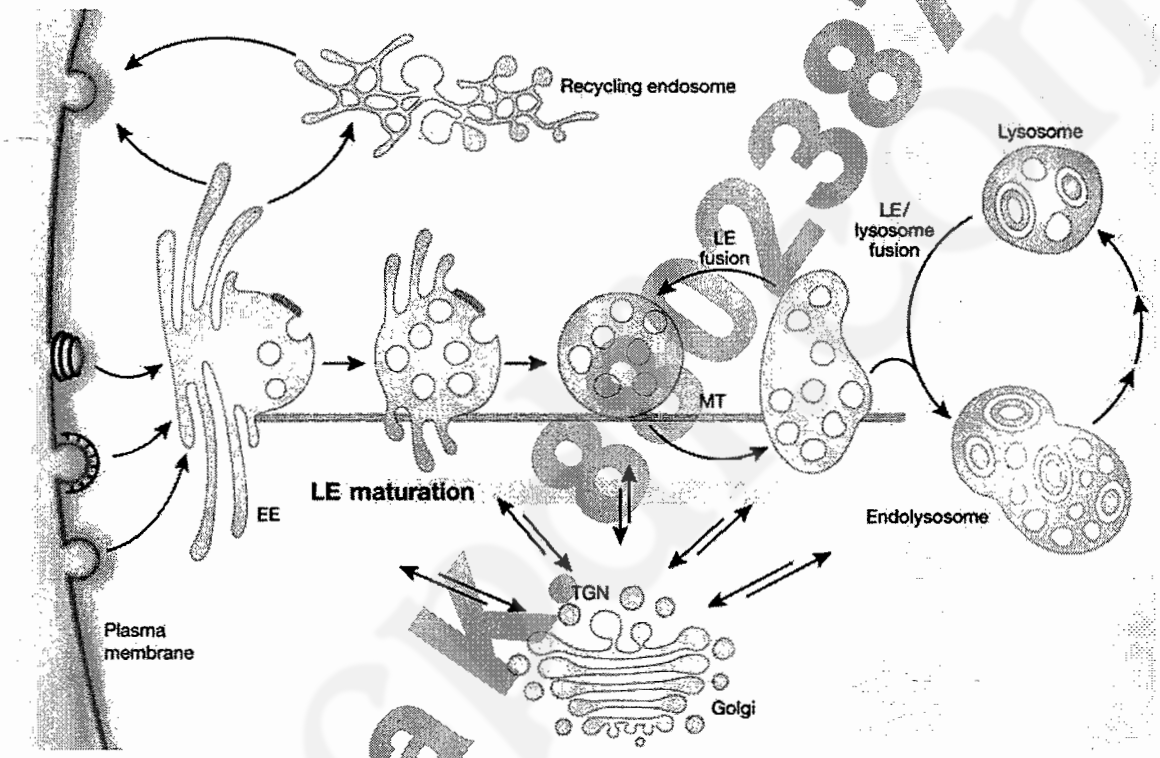


Figure 13: The endosome/ lysosome system.

Functions of endosomes

An endosome is a compartment of the endocytic membrane transport pathway from the plasma membrane to the lysosome. Molecules internalized from the plasma membrane can follow this pathway to lysosomes for degradation.

Many materials can be recycled back to the plasma membrane.

Molecules are also transported to endosomes from the Golgi and either continue to lysosomes or recycle back to the Golgi.

Furthermore, molecules can be directed into vesicles that bud from the perimeter membrane into the endosome lumen.

Therefore, endosomes represent a major sorting compartment of the endomembrane system in cells.

PLANT PEROXISOMES

Introduction

Peroxisomes, also termed microbodies, are small, spherical organelles in most eukaryotic cells. They are enclosed by only a single membrane and have a diameter of 0.5 to 1.5 μm. Peroxisomes were discovered and characterized by **Christian de Duve** and co-workers in 1955.

They participate in a wide variety of essential metabolic pathways in nearly all eukaryotes. These multipurpose organelles contain enzymes for many physiological reactions, including the production of hydrogen peroxide, the β-oxidation of long-chain fatty acids, and in some organisms, the synthesis of cholesterol or penicillin. A shared feature of all peroxisomes is their ability to metabolize hydrogen peroxide, thus protecting the rest of the cell from this toxic byproduct.

Occurrence and Localization

Peroxisomes are a common constituent of eukaryotic cells.

In plants there are three important differentiated forms:

1. the **leaf peroxisomes**, which participate in photorespiration;
2. the **glyoxysomes**, which are present in seeds containing oils (triacylglycerols). They play a role in the conversion of triacylglycerols to carbohydrates. They contain all the enzymes for fatty acid β-oxidation.
3. the **nitrogen metabolizing peroxisomes**, which are located in uninfected cells of root nodules. They contain enzymes, including uricase and allantoinase, that assist in nitrogen metabolism.

Within a cell, they are mainly found in the peripheral regions, just behind the cell cortex. In the leaves of C3 plants, they are attached to chloroplasts and mitochondria. This allows easy metabolite transfer during photorespiration.

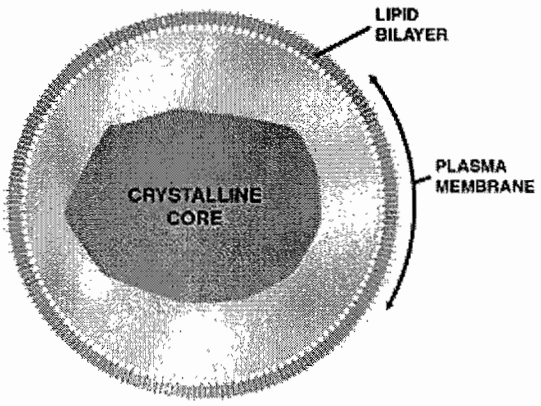


FIGURE 14: PEROXISOME STRUCTURE

In the recent years, it has been reported that peroxisomes alter their abundance and location in response to physiological and environmental factors. In 2002, Jaideep Mathur and co-workers (at University of Köln, Germany) showed that actin (and not microtubule as earlier believed) governs peroxisome motility in plant cells.

Structure

The peroxisomes have a diameter of 0.5 to 1.5 □m with spherical shape. They are enclosed by only a single membrane. They are viewed best by transmission electron microscopy.

The single membrane of peroxisomes encloses a granular matrix filliing the inner space. The enzymes in the matrix usually occur at very high concentrations forming a crystalloid core. This crystalline core enables easy detection through electron microscopy, as shown in Figure 1. In peroxisomes, after disruption of the boundary membrane, the peroxisomal matrix proteins remain aggregated in the form of particles of a size similar to peroxisomes, and the compartmentalization of the peroxisomal reactions is maintained (Goodman, 1992).

Peroxisomes do not possess their own genome. All peroxisomal proteins are nuclear encoded, synthesized on free cytosolic ribosomes, and imported posttranslationally into the organelle.

Biochemical composition

The peroxisomal matrix represents a specialized compartment for reactions in which toxic intermediates are formed.

This membrane also contains porins. Peroxisomes contain **enzymes catalyzing the oxidation of substances accompanied by the formation of H_2O_2**, and also contain **catalase**, which immediately degrades this H_2O_2. Differentiated peroxisomes also contain **enzymes for fatty acid □-oxidation**, and enzymes like **uricase** and **allantoinase**. **Enzymes involved in jasmonic acid biosynthesis** (Stintzi and Browse, 2000) and **the metabolism of reactive oxygen species** also have recently been discovered in plants (Corpas et al., 2001).

Functions

Physiological functions of peroxisomes vary depending on the type of tissue in which they are found, and also on the metabolic and developmental state of the organism. Thus, plants have several classes of peroxisomes, each present at different stages in the life cycle and each having different set of enzymes specific for the physiological role of the organelle. The interconversion between peroxisome classes appears to be transcriptionally regulated; the mRNA expressed at a given time in a given tissue determines which enzymes are present in the organelle (Olsen, 1998).

Well characterized important functions of peroxisomes are summarized below:

1. **The role in photorespiration:** This role is played by the leaf type peroxisomes in plants. In this process, two other organelle are also involved, namely the chloroplast and mitochondria. The photorespiratory process and the position of peroxisomes in it are depicted below in Figure 2.

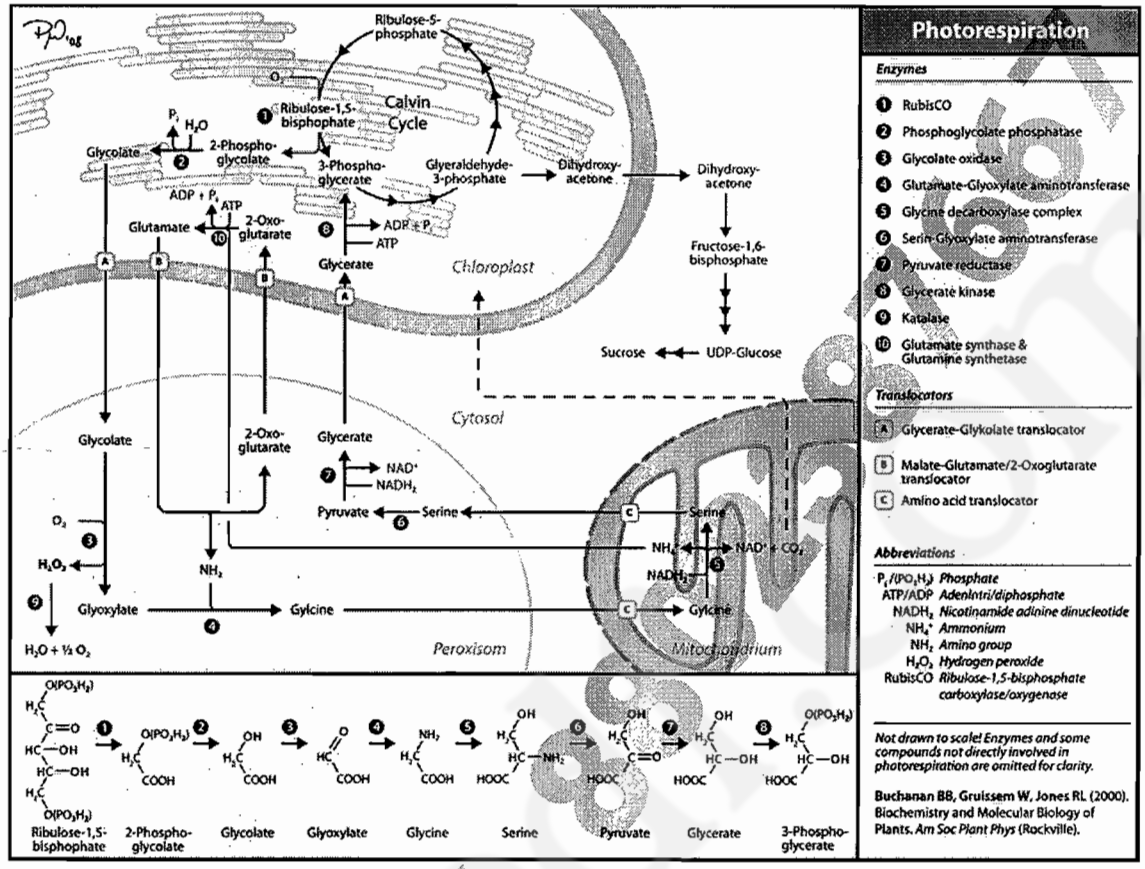


Figure 15: THE PHOTORESPIRATORY PROCESS

During the conversion of glycolate to glycine, two toxic intermediates are formed: glyoxylate and H_2O_2. In isolated chloroplasts, photosynthesis is completely inhibited by the addition of low concentrations of H_2O_2 or glyoxylate.

Compartmentalization of the conversion of glycolate to glycine and conversion of H_2O_2 to Water in the peroxisomes serves the purpose of eliminating the toxic intermediate products glyoxylate and H_2O_2 at the site of their formation, so that they do not invade other cell compartments.

2. **The role during seed germination:** In germinating oil seeds, storage lipids are mobilized for the production of carbohydrates in the glyoxysomes, which are specialized peroxisomes. At the beginning of germination, storage proteins are degraded to amino acids, from which the enzymes required for the mobilization of the storage lipids are synthesized. These enzymes include lipases, which catalyze the hydrolysis of triacylglycerols to glycerol and fatty acids. Lipases bind to the membrane of oleosomes. The glycerol formed by the hydrolysis of triacylglycerol, after phosphorylation to glycerol 3-phosphate and its subsequent oxidation to dihydroxyacetone phosphate, can be fed into the gluconeogenesis pathway. The released free fatty acids are first activated as CoA-thioesters and then degraded to acetyl CoA by β-oxidation (Fig. 3). Acetyl-CoA is then utilized for the glyoxylate cycle.

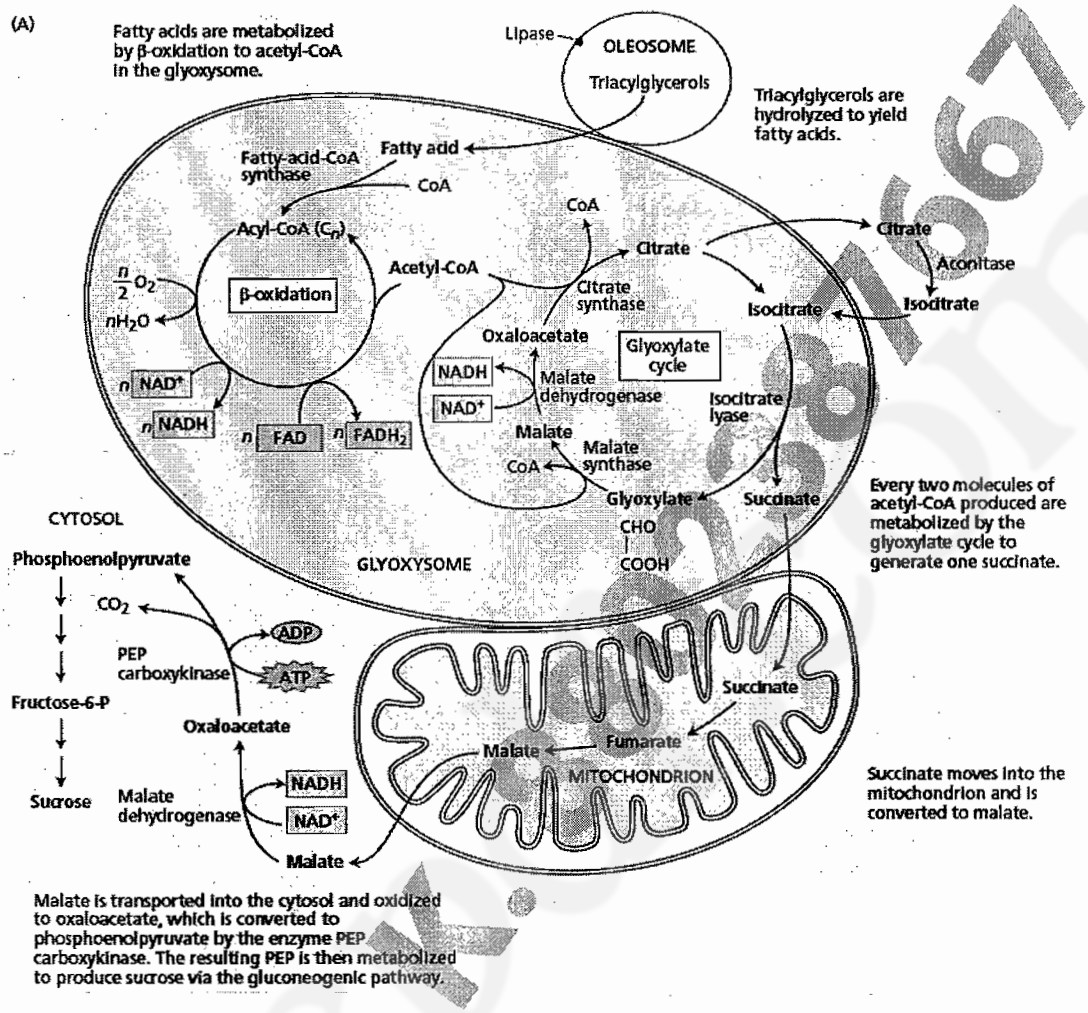


Figure 16: The role of glyoxysomes in germinating oil seeds

β-oxidation of Fatty Acids

The peroxisomes in yeast and plant cells carry out the breakdown of fatty acid molecules by *β oxidation*. In this pathway the alkyl chains of fatty acids are shortened sequentially by blocks of two carbon atoms at a time, thereby converting the fatty acids to acetyl CoA. In mammalian cells, β oxidation occurs in both mitochondria and peroxisomes;, however, this essential reaction in plants and yeasts occurs exclusively in peroxisomes.

Glyoxylate Pathway

It is a metabolic pathway present in plants and some bacteria that converts two-carbon acetyl units into four-carbon units (succinate) for energy production and biosyntheses. This reaction sequence, called the *glyoxylate cycle*, bypasses the two decarboxylation steps of the citric acid cycle. Another key difference is that two molecules of acetyl CoA enter per turn of the glyoxylate cycle, compared with one in the citric acid cycle.

The steps of this cycle are:

1. The glyoxylate cycle begins with the condensation of acetyl CoA and oxaloacetate to form citrate by *Citrate Synthase*.
2. Citrate is then isomerized to isocitrate by *Aconitase*.
3. Isocitrate is cleaved by *isocitrate lyase* into succinate and glyoxylate. Succinate thus generated is used for for energy production and biosyntheses. The subsequent steps regenerate oxaloacetate from glyoxylate.
4. Acetyl CoA condenses with glyoxylate to form malate in a reaction catalyzed by *malate synthase*, which resembles citrate synthase.
5. Finally, malate is oxidized to oxaloacetate, as in the citric acid cycle.

The sum of these reactions is:

$$2\,\text{Acetyl CoA} + \text{NAD}^+ + 2\,H_2O \longrightarrow \text{succinate} + 2\,\text{CoA} + \text{NADH} + 2\,H^+$$

In plants, succinate can be converted into carbohydrates by a combination of the citric acid cycle and gluconeogenesis. Thus, organisms with the glyoxylate cycle gain a metabolic versatility.

3. **The role in Detoxification:** Peroxisomes are so named because they use molecular oxygen to remove hydrogen atoms from specific organic substrates (designated here as R) in an oxidative reaction. And this oxidative reaction produces hydrogen peroxide (H_2O_2): $RH_2 + O_2 \rightarrow R + 2H_2O_2$

   Catalase utilizes the H_2O_2 generated in the organelle as an outcome of the above reaction. The reaction of peroxide breakdown is: $2\,H_2O_2 \rightarrow 2H_2O + O_2$

   Catalase also oxidizes a variety of other substrates – including phenols, formic acid, formaldehyde, and alcohol—by the peroxidative reaction: $H_2O_2 + R'\,H_2 \rightarrow R' + 2H_2O$.

   This type of oxidative reaction is particularly important in animal liver and kidney cells, where the peroxisomes detoxify various toxic molecules that enter the bloodstream. For example about 25% of the ethanol is oxidized to acetaldehyde in this way. Moreover, when excess H_2O_2 accumulates in the cell, catalase converts it to H_2O through the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$. Prokaryotes lack peroxisomes, so they are more vulnerable to toxic substances like hydrogen peroxide.

4. **The role in plant stress response:** Enzymes involved in jasmonic acid biosynthesis (Stintzi and Browse, 2000) and the metabolism of reactive oxygen species recently have been discovered in plants (Corpas et al., 2001), suggesting that peroxisomes play important roles in various biotic and abiotic stress responses.
5. **Role in Nitrogen Metabolism:** Peroxisomes in uninfected cells of root nodules contain enzymes, including uricase and allantoinase, that assist in nitrogen metabolism (Webb and Newcomb, 1987).

6. **Role in Fatty Acid Synthesis:** In 2004, Yun Lin and co-workers reported that the peroxisome deficient *Arabidopsis* mutant *sse1* (*Shrunken Seed 1*) exhibits impaired fatty acid synthesis. The ability of *sse1* to incorporate oleic acid, but not pyruvate or acetate, into triacylglycerol indicated a defect in the fatty acid biosynthetic pathway in this mutant. The results point to a possible role for peroxisomes in the net synthesis of fatty acids in addition to their established function in lipid catabolism.

7. **Biosynthetic functionin Animal Cells:** Animal peroxisomes catalyze the first reactions in the formation of *plasmalogens*, which are the most abundant class of phospholipids in myelin. Deficiency of plasmalogens causes profound abnormalities in the myelination of nerve cells, which is one reason why many peroxisomal disorders lead to neurological disease.

Maintenance and Multiplication

Peroxisomes usually self-replicate by enlarging and then dividing. Recently there is some indication that new peroxisomes may be formed directly.

For their functional maintenance the peroxisomes import all their protein from the cytosol because they have no genome like mitochondria or chloroplasts. The protein import into the peroxisomes uses the non-secretory pathway.

A short signal sequence of three amino acids directs the import of proteins into peroxisomes. The process of protein transport into the peroxisomes involves soluble receptor proteins in the cytosol that recognize the targeting signals. Proteins called **peroxins** (**Pex**) also participate as components in the process.

The importance of this import process and of peroxisomes is demonstrated by the inherited human disease ***Zellweger syndrome***, in which a defect in importing proteins into peroxisomes leads to a severe peroxisomal deficiency. This disease is caused by a mutation in the gene encoding a peroxisomal integral membrane transport protein, the **peroxin Pex 2**. These individuals, whose cells contain "empty" peroxisomes, have severe abnormalities in their brain, liver, and kidneys, and they die soon after birth.

NUCLEUS

The presence of the nucleus is the fundamental feature that distinguishes eukaryotic from prokaryotic cells. As a matter of fact, the term eukaryote means "true nucleus" (*eu* = true or well developed; *karyon* = nucleus). It was scientifically characterized and named by **Robert Brown** in 1830s.

What is a nucleus?

The nucleus is an enveloped cellular compartment of all eukaryotic cells, visible only during the interphase of the cell cycle, which contains the genomic DNA and serves as the site of initial stages of gene expression. Since all the types of genome expression begin in the nucleus, which ultimately govern the entire range of cellular functions – it is also commonly referred to as **the control centre of the cell**. The classical experiment by Hammerling on *Acetabularia* showed that the nucleus determines the characters of the cell and ultimately of the individual. In 1997, researchers at the Roslin Institute of Scotland announced that they had successfully cloned a sheep (**Dolly**) by using the genetic material from a differentiated cell of an adult animal by the technique of nuclear transplant. This also establishes the nucleus as the control centre of the cell.

Some specialised types of cells, such as mature RBCs and phloem sieve elements in plants, do not contain a nucleus and hence they never divide. Cell can carry out some specialised functions in the absence of a nucleus but will never reproduce without it. In 1928, Spemann showed that the enucleated part of a zygotic cell does not divide or have any developmental future.

Nuclear structure

Gross Structure

Under proper staining, the nucleus can be conveniently studied even with a light microscope. In an animal cell the nucleus is the largest compartment, while in the plant cells it is the second largest compartment after the vacuole.

Depending on the cell type, the number of nucleus per cell can vary widely. Some examples include:

1. Mature RBCs in mammals do not have a nucleus
2. Mature sieve element cells in plants do not have a nucleus
3. *Paramecium* is binucleate

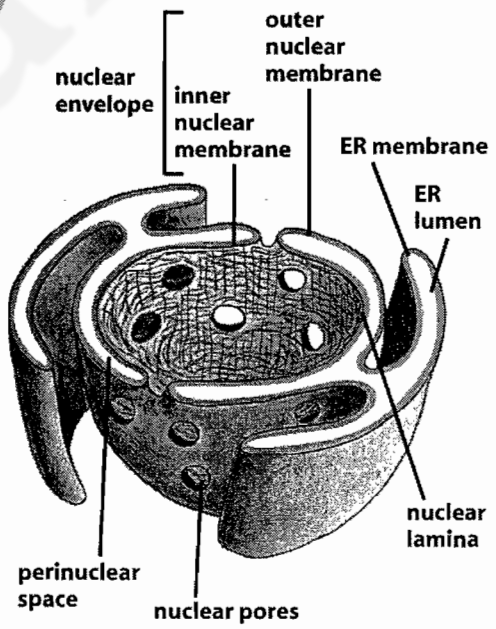


FIGURE 1: Nucleus: The Gross Organization

4. The cells of Cartilage and Liver are binucleate
5. Dikaryon is a well established state in the life-cycle of the fungi belonging to the Ascomymycota and Basidiomycota
6. Syncytial cells are fairly common among the animals, such as Trophoblast of the Human Embryo, Skeletal Muscle Fibers etc.
7. Coenocytic stage is also common in plants, algae and fungi – such as Amoeboid Tapetal Mass in angiosperms, the thallus of the alga *Vaucheria* and the thallus of the fungi from Oomycota.

The shape is mostly oval or spherical but it can vary. In most cells, the nucleus is spherical or oblong, which minimizes the surface area needed to enclose a specific volume. The percentage of total cell volume occupied by nuclei of different types of cells varies widely, from 1–2 percent in yeast cells, to 10 percent in most somatic cells, to as much as 40–60 percent in cells that have less need for cytoplasmic functions such as secretion.

In terms of **Size**, the nucleus is largest visible component of the animal cell under the light microscope. The diameter ranges from about one micron (1 μm) to more than 10 μm. The size of the nucleus is related to the amount of DNA it contains. Hence, polyploid cells have bigger nuclei. The nuclei of the oocytes of the frog *Xenopus laevis* have a diameter of ~400 μm, due to which it is widely used in cell biological and biochemical studies.

The relation of nuclear volume to the cytosolic volume is given by the **Hertwig's relation**:

NP = Vn / Vc-Vn [Where NP = Nucleoprotein Index, Vn= Volume of the nucleus, Vc = Volume of the cytoplasm.

Fine Structure

A combination of Electron Microscopy and sophisticated biochemical analysis reveals that the nucleus has the following components:

1. The **nuclear envelope** has two concentric membranes (inner and outer nuclear membranes) that surround the nucleus and its underlying intermediate filament lattice, the **nuclear lamina**. The nuclear envelope is penetrated by nuclear **pores**. The outer membrane is continuous with the membrane of the rough endoplasmic reticulum. A **nuclear pore complex (NPC)** is a very large, proteinaceous structure that extends through the nuclear envelope, providing a channel for bidirectional transport of molecules and macromolecules between the nucleus and the cytosol.

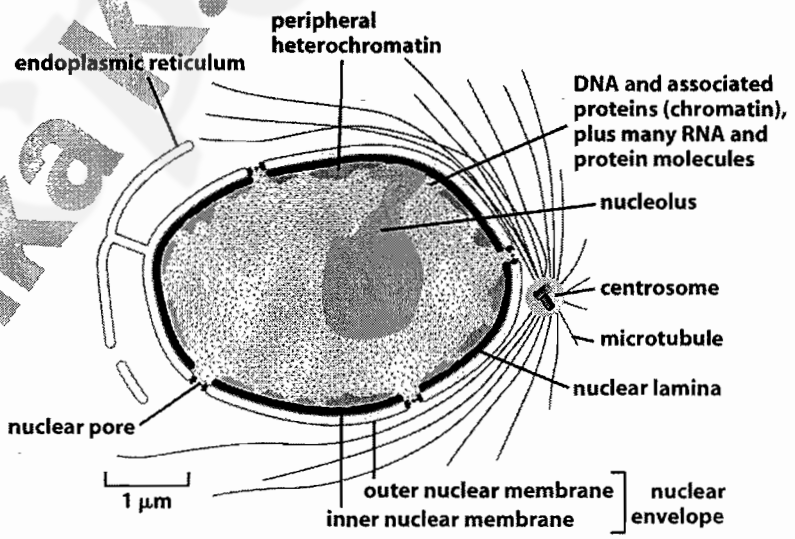


FIGURE 2: Fine Organization of the Nucleus. Please also refer to your class lecture illustration.

2. The **nucleolus** (*plural:* nucleoli) is a discrete region of the nucleus where ribosomes are produced.
3. **Nuclear matrix** which is a filamentous network to which the chromatin material attach.
4. The **nucleoplasm** refers to the content of the nucleus, excluding the nucleolus. The nucleoplasm contains **Replication Factories** and **Transcription Factories** [Scheer *et al*, 2004]
5. **Heterochromatin** describes regions of the genome that are highly condensed, are not transcribed, and are late-replicating. Heterochromatin is divided into two types, which are called constitutive and facultative.
6. **Euchromatin** comprises all of the genome in the interphase nucleus except for the heterochromatin. The euchromatin is less tightly coiled than heterochromatin, and contains the active or potentially active genes.

Nuclear Envelope and Nuclear Pore Complexes

Nuclear Envelope [also called Perinuclear Cisterna in older literature] is seen only in electron micrographs. The nuclear envelope has a complex structure, consisting of two nuclear membranes [each 7.5 – 8 nm thick], an underlying nuclear lamina, and nuclear pore complexes. The two membranes are separated by a lumen or the Perinuclear Space (10-40 nm wide) that is contiguous with the endoplasmic reticulum (ER) lumen.

The nuclear membranes have standard biomembrane structure comprising a core of of glycerophospholipid bilayer and integral, anchored and peripheral proteins.

The nuclear lamina is a fibrous meshwork underlying the inner nuclear membrane. It provides structural support to the nucleus. The nuclear lamina is composed of proteins called lamins. Most mammalian cells contain four different lamins, designated A, B1, B2, and C. All the lamins are 60- to 80-kilodalton fibrous proteins, related to the intermediate filament proteins of the cytoskeleton. Lamin proteins are called intermediate filament proteins because the size of the filaments they form (10–20 nm in diameter) is intermediate between that of actin microfilaments (7 nm in diameter) and that of microtubules (25 nm in diameter). The nuclear lamina is 10–20 nm thick and is interrupted by nuclear pore complexes, which are anchored to the nuclear lamina.

The association of lamins with the inner nuclear membrane is facilitated by prenyl groups and the binding to a set of integral membrane proteins called lamina-associated proteins (LAPs).

No nuclear lamins are encoded in plant genomes, but plants may contain other structural proteins that function like lamins in animal cells. Yeasts (such as S. cerevisiae and S. pombe) and some other unicellular eukaryotes lack lamins and therefore have no lamina.

In addition to its roles in nuclear reassembly and structural support, the nuclear lamina interacts with chromatin and may be needed for DNA replication to occur. The lamins bind chromatin through nuclear matrix proteins.

The nuclear pore complexes (NPCs) are the channels that span the nuclear envelope and through which macromolecules like proteins and RNA pass between the nucleus and cytoplasm.

The nuclear envelopes of most cells contain about 10–20 NPCs per square micrometer of surface. Thus, yeast cells have 150–250 NPCs, whereas mammalian somatic cells contain 2,000–4,000. Some cells,

however, have a much greater density of NPCs, most likely because the cells are very active transcriptionally and translationally, requiring transport of many macromolecules into and out of the nucleus. For example, the nuclear surface of amphibian oocytes is almost completely covered by NPCs.

The nuclear pore complex is an extremely large structure with a diameter of about 120 nm and an estimated molecular mass of approximately 50 million (10^6) daltons—about 15 times the size of a ribosome. The nuclear pore complex is composed several different proteins, belonging to the family **nucleoporins**. In a recent study published in the 29 November 2007 issue (Vol 450) of Journal *Nature* , Frank Alber, Svetlana Dokudovskaya, Liesbeth M. Veenhoff *et al* have established that each NPC contains at least 456 individual peptide molecules and is composed of ~30 distinct proteins (nucleoporins).

In cross section, a nuclear pore complex seems to have five structural building blocks (Fig. 3):

1. column subunits, which form the bulk of the pore wall;
2. annular subunits, which extend "spokes" (not shown in Fig. 3) toward the center of the pore;
3. lumenal subunits, which contain transmembrane proteins that anchor the complex to the nuclear membrane; and
4. ring subunits, which form the cytosolic and nuclear faces of the complex.
5. a central pore (not shown in Fig. 3)

In addition, fibrils protrude from both the cytosolic and the nuclear sides of the complex. On the nuclear side, the fibrils converge to form basketlike structures. The proteins that make up the core of the nuclear pore complex are symmetrically distributed across the nuclear envelope so that the nuclear and cytosolic sides look identical.

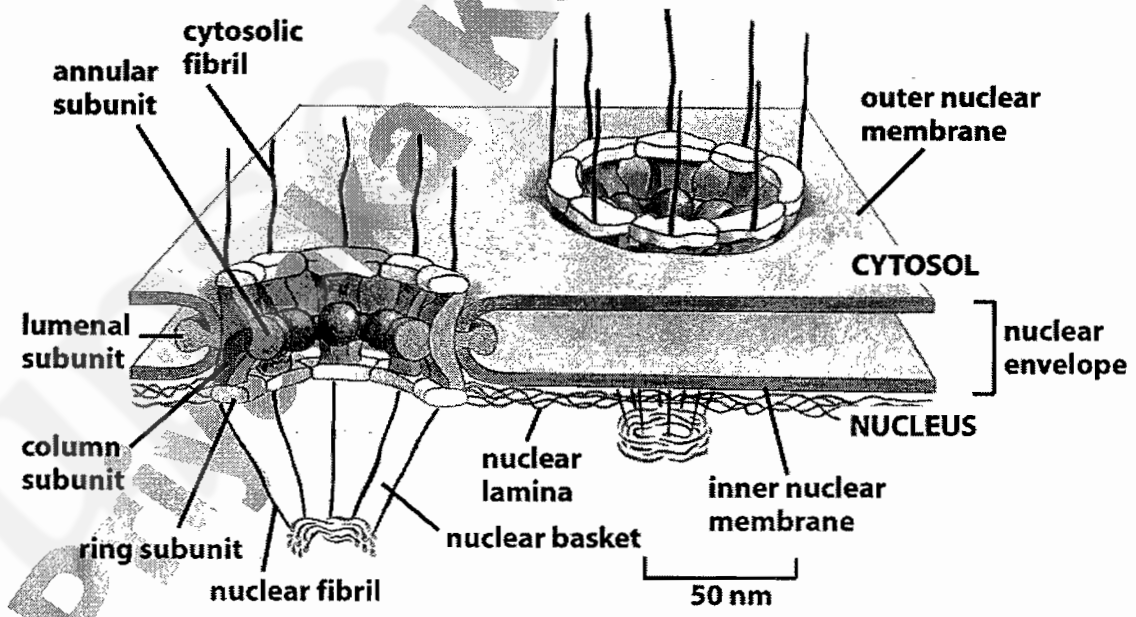


FIGURE 3: Fine Structure of the Nuclear Pore Complex

Visualization of nuclear pore complexes by electron microscopy reveals a structure with eightfold symmetry organized around a large central channel, which is the route through which proteins and RNAs cross the nuclear envelope. Detailed structural studies, including immunoelectron microscopy and computer-based image analysis by Pante' and Aebi in 2001, have led to the development of three-dimensional models of the nuclear pore complex. Electron microscopy studies in several organisms have revealed that the general morphology of the NPC is conserved.

These studies indicate that the nuclear pore complex consists of an assembly of eight and eight large proteins forming cytosolic surface and nuclear surface rings each; and eight spokes arranged radially around a central channel that serves as the conduit for macromolecular transport. The spokes are connected to annular sububits and separated by an average distance of 10 nm. The NPC assembly is anchored within the nuclear envelope at sites of fusion between the inner and outer nuclear membranes. Protein filaments extend from both the cytoplasmic and nuclear rings, forming a distinct basketlike s ıe nuclear side.

The central channel is maximum 40 nm in diameter, which is wide enough to accommodate the largest particles able to cross the nuclear envelope. But, in the resting stage it is only 10 nm wide. It contains a structure called the central transporter, through which the active transport of macromolecules is thought to occur.

The following table summarises the flux of macromolecules through the NPC.

Table 1: ***THE FLUX OF MACROMOLECULES THROUGH THE NPC***

Direction	Substance	Molecules passed / Minute
Import	Histones	100
Import	Non Histone Proteins	100
Import	Ribosomal Proteins	150 - 200
Export	Ribosomal Subunits	5 - 8
Export	mRNA	1-5, can vary widely

Correlation of Molecular Weight & Nuclear Transport Strategy:

1. Very low, such as CO_2, O_2 and H_2O: by simple diffusion
2. Less than 8 Kd: Can pass through the spokes, or resting translocator
3. 8-20 Kd: Can pass easily but requires NLS or NES
4. 20-50 Kd: Passes slowly and requires NLS or NES
5. More than 50 Kd: Would not pass at all.

Nuclear Matrix and Nucleoplasm

The localization of nuclear processes to discrete sites suggests that there may be an underlying structure in the nucleus. Nuclei do not contain a highly ordered skeleton resembling the cytoskeleton. However, some recent studies suggest that a sort of filamentous network, called the nuclear matrix, may exist in the nucleus. In contrast to the easily visualized cytoskeleton, this network is seen only if nuclei are treated with detergents, DNase, and high salt concentration. **The nuclear matrix network contains short fibers approximately the size of intermediate filaments, actin (not in its filamentous form), and many other**

proteins (He, Nickerson, and Penman, 2002). These components are not well organized into any larger structure. Because the nuclear matrix is relatively insoluble, it is difficult to study it as a whole.

Within the nuclear matrix, there are certain specific locations where the chromatin materials are found. These locations are called the **chromatin domain**. The chromatin attaches to the nuclear matrix through specialised regions called **Matrix Association Regions (MARs)**.

The locations where there is no chromatin material are regarded as the **interchromatin domain**. The interchromatin domain is equivalent to the **nucleoplasm**, which contains a vast array of enzymes and regulatory proteins. The **Replication Factories** and **Transcription Factories** are also found in the nucleoplasm.

The nucleoplasm is transparent, semi-solid, granular and slightly acidophilic ground substance. It is also known as **karyolymph**.

The nucleoplasm has a complex chemical composition. It is composed of mainly the nucleoproteins but it also contains other inorganic and organic substances, *viz.,* ribonucleic acids, structural proteins, enzymes and minerals.

The nucleoproteins can be categorized into following two types:

(i) **Basic proteins.** The proteins which take basic stain are known as the basic proteins. The most important basic proteins of the nucleus are **nucleoprotamines** and the **nucleohistones.**

The nucleoprotamines are simple and basic proteins having very low molecular weight (about 4000daltons). The most abundant amino acid of these proteins is arginine (*p*H 10 to 11). The protamines usually remain bounded with the DNA molecules by the salt linkage. The protamines occur in the spermatozoa of the certain fishes.

The nucleohistones have high molecular weight, *e.g.,* 10,000 to 18,000 daltons. The histones are composed of basic amino acids such as arginine, lysine and histidine. The histone proteins remain associated with the DNA by the ionic bonds and they occur in the nuclei of most organisms. According to the composition of the amino acids following types of histone proteins have been recognised,e.g., histones rich in lysine, histones with arginine andhistones with poor amount of the lysine.

(ii) **Non-histone or acidic proteins.** The acidic proteins either occur in the nucleoplasm or in the chromatin. The most abundant acidic proteins of the euchromatin (a type of chromatin) are the phosphoproteins.

ENZYMES. The nucleoplasm contains many enzymes necessary for the synthesis of the DNA and RNA. The most important nuclear enzymes are the DNA polymerase, RNA polymerase, NAD synthetase, nucleoside triphosphatase, adenosine-diaminase, nucleoside-phosphorylase, guanase, aldolase, enolase, 3-phosphoglyceraldehyde dehydrogenase and pyruvate kinase. The nucleoplasm also contains certain cofactors and coenzymes such as ATP and acetyl CoA.

LIPIDS. The nucleoplasm contains small lipid content.

MINERALS. The nucleoplasm also contains several inorganic compounds such as phosphorus, potassium, sodium, calcium and magnesium. The chromatin comparatively contains large amount of these minerals than the nucleoplas.

Please refer to your Class Lecture Notes and Diagrams for a description of:

1. The Nuclear Matrix
2. The nucleolus (*plural:* nucleoli)
3. Replication Factories and Transcription Factories
4. Heterochromatin and Euchromatin

Advantages of having a nucleus

1. The nucleus protects the DNA of the cell from exposure to various DNA damaging agents and mutagens.
2. The nucleus also allows for sophisticated gene regulation by ensuring a spatial as well as a temporal gap between transcription and translation.
3. The dynamic cytoskeleton of the eukaryotic cells generates shear forces that could break the DNA, were it not protected within the nucleus during interphase.
4. During mitosis, chromosomes become more compact, and the DNA is folded into a highly ordered structure. In the condensation of the chromatin material the nuclear matrix proteins play a critical role.

NUCLEOLUS

Introduction

Most eukaryotic cells [except certain Dinoflagellates] **during the interphase** contain dense and acidophilic sub-compartments within the nucleus clearly visible even with a light microscope. They are found associated with the **Nucleolar Organizer Region** (NOR) of specific chromosomes as dark staining knobs and are the sites of rRNA transcription and the biogenesis of ribosomal sub-units. These nuclear sub-compartments have been designated as **Nucleolus**. In addition to its role in ribosomal RNA biogenesis it has been implicated in control of cellular survival and proliferation.

Occurrence

The Nucleolus is found in almost all the eukaryotic nuclei. It is probably the most striking and well studied sub-compartment of nucleus. It is visualized even with a light microscope when acidophilic stains are appropriately applied. Mammalian nuclei usually contain between 1-4 nucleoli and in *Zea mays* there are two nucleoli, associated with chromosome 6 and 9.

Ultrastructure

By applying cryofixation methods, it is possible to identify morphological sub compartments in the nucleolus of *S. cerevisiae* that are similar to those of nucleoli of higher eukaryotes.

The nucleolus consists of three morphologically distinct components:

1. **The fibrillar centres (FC),** which contain hundreds of rRNA genes in tandem arrays found at nucleolar organising regions(NORs). Mammalian nucleoli typically contain several fibrillar centres (FC)
2. **The dense fibrillar component (DFC),** which contains actively transcribing rRNA genes and nascent rRNA transcripts; and
3. **The granular component (GC),** which is the site of late processing events in the biogenesis of rRNAs

The nucleolus is not a fixed structure. Metazoan nucleoli are highly dynamic and undergo rounds of disassembly and reassembly during the cell cycle. Upon entry into mitosis, the nucleolus disintegrates in the prophase and reappears in the Telophase. During the M-phase, the nuclear disassembly is triggered by CDK1+Cyclin B complex. However, throughout the interphse, the nucleolus is stable.

Recently discovered sub-nucleolar domains

Recent works reveal that there are several sub-nucleolar domains associated with nucleoli. Most importantly they include:

1. **Perinucleolar compartment:** involved in multiple aspects of RNA metabolism and rRNA processing. (Wang et al., 2003).
2. Cajal bodies: often found in close proximity to nucleoli. Cajal bodies play a role in snRNP biogenesis and in the trafficking of snoRNPs and snRNPs.
3. **Sam68 bodies:** involved in RNA metabolism
4. **SPECKLES:** are the centres of various RNA peocessing enzymes.
5. **Paraspeckles:** play a role in rRNA processing and splicing in the GC region of nucleolus.
6. **GEMINI BODIES** are the centres of mRNA modifications / editing etc.

Biochemistry

The nucleolus is largely constructed of RNA and proteins. Through the use of mass spectrometry, many hundred protein components of the nucleolus have been identified. **The analysis of these proteins highlights many biological functions and pathways** operating within the nucleolus including,

1. rRNA formation and ribosome biogenesis
2. mRNA metabolism
3. genesis of translation factors
4. genesis of chaperones
5. genesis of cell cycle regulators
6. genesis of components of signal recognition particles (SRP).

Role in Ribosome biogenesis

The synthesis of the eukaryotic ribosome is a complex phenomenon, involving several regions of the cell. **The NOR of eukaryotic chromosomes contains the genes for the 18S, 5.8S and 28S rRNAs and the closely associated nucleolus has the transcription machinery for these genes.** The 18S, 5.8S and 28S rRNAs are synthesized as a part of a much longer precursor molecule in the nucleolus. The NOR contains multiple rRNA genes arranged in tandem repeats along the DNA molecules separated from each other by non-transcribing spacer DNA stretches. **The 5S rRNA is synthesized outside the NOR.**

The three rRNA genes are transcribed together as a long precursor 45S RNA. The precursor later undergoes splicing and processing with aid

of Nucleolin, U3 protein, and snRNP. As mentioned earlier, the 5S rRNA is synthesized outside the NOR. About 70 different types of proteins are involved in ribosome assembly, which all are synthesized in the cytoplasm. All these components of ribosome migrate to the nucleolus, where they are assembled into 40S and 60S ribosomal subunits. The 40S ribosomal subunit is assembled and migrates the nucleus earlier than the 60S ribosomal subunit. This time lag is important to ensure that unprocessed hnRNA are not exposed to functional ribosomes; a situation that can synthesize wrong proteins.

The assembly of ribosomal subunits is a centrifugal process, which means that:

1. **the initiation** , i.e. the rRNA transcription takes place in the FC
2. **the production**, i.e. the rRNA + protein assembly occurs in the DFC
3. **the maturation,** which is processing and precise 3-D alignment of the ribosomal subunits, takes place in the GC.

Other roles

Beyond its role in ribosomal RNA biogenesis, the nucleolus appears to act as a storage site or reservoir for a number of proteins that do not have roles in rRNA metabolism. The nucleolar localization of proteins appears to be a common means of cell cycle regulation among eukaryotes. For example, the tumour suppressor ARF, is a nucleolar protein. Nucleolar proteins are known to be mutated in a number of disease including TCOF in Treacher Collins and ataxin 7 in a form of spinocerebellar ataxia. In addition, several proteins that localise to the nucleolus are involved in cancer including both the Werner's syndrome (WRN) and the Bloom's syndrome (BLM) gene products, which are DNA helicases that affect genome stability.

CHROMOSOMES

What are chromosomes?

A chromosome is discrete physical unit of genetic transmission, which essentially is a nucleoprotein complex comprising of a single molecule of nucleic acid and associated structural proteins with a special organization, structure, individuality and dynamism. The chromosomes are not only central to genetic transmission but also play a fundamental role in mutation, recombination and evolution.

The way the term chromosome is defined has changed over years, as our scientific understanding on the organization of genomes in various biological entities has refined in the wake of new findings.

In older literature, the term chromosome was used only in the context of eukaryotic genomes. The prokaryotes and viruses were supposed to have naked genetic material. However, we know today that the bacteria or the viruses have no naked genetic material. They rather have nucleic acids which is complexed with structural proteins in a very specific manner. As a result, in current genetic or molecular biological literature we find the mention of bacterial chromosome or a viral chromosome.

Therefore, to summarize, our current understanding on the organization of genomes makes us define the chromosomes in a wider manner. Accordingly, in the biological world we have the following types of chromosomes in terms of molecular organization.

Type	Brief Description
Viral	• Genetic material DNA or RNA, ss or ds, Linear or Circular • Complexing protein is the capsid protein • The genetic material interacts with the capsid protein through specific Capsid Association Regions (CARs)
Bacterial	• Genetic material always dsDNA with mostly circular geometry (but *Borrelia burgdorferi* and *Streptomyces coelicolor* have linear dsDNA). • The complexing proteins are 3 in total: 1. HLP: small molecular weight, bind to DNA through minor grooves as regular intervals 2. HU: a dimeric protein (with huA and huB units) that binds to DNA and condenses it in bead like structure; also known to stimulate DNA replication. 3. H1: also called H-NS, preferentially binds to bend DNA regions
Eukaryotic - I	• Genetic material always ds DNA with linear geometry • The 1° complexing protein is **not** histone but some other basic protein, such as: 1. Protamines: found in higher vertebrate sperm cells 2. Small molecular weight basic proteins binding to the genomic DNA of Dinoflagellates
Eukaryotic - II	• Genetic material always ds DNA with linear geometry • The 1° complexing protein is **always** histone as we find in the genomes of most of the eukaryotes

History of chromosomes

Chromosomes were first observed in plant cells by Swiss botanist Karl Wilhelm von Nägeli (1917-1891) in 1842, and independently, in *Ascaris* worms, by the Belgian scientist Edouard Van Beneden (1846-1910). The use of basophilic aniline dyes was a fundamentally new technique for effectively staining the chromatin material inside the nucleus. Their behavior in animal (salamander) cells was later described in detail by German anatomist Walther Flemming (1843-1905), the discoverer of mitosis, in 1882. The name was invented later by another German anatomist, Heinrich von Waldeyer. In 1910, American geneticist Thomas Hunt Morgan (1866-1945) proved that chromosomes are the carriers of genes, by studying the common fruit fly (*Drosophila melanogaster*).

GROSS ATTRIBUTES of the chromosome set

Number of Chromosomes

Different species have highly characteristic chromosome numbers, and examples are shown in the Table 1 below. The range is immense, from two in some flowering plants to many hundreds in certain ferns.

Table 1. NUMBERS OF PAIRS OF CHROMOSOMES IN DIFFERENT SPECIES OF PLANTS AND ANIMALS

COMMON NAME	SPECIES	NUMBER OF CHROMOSOME PAIRS
Mosquito	*Culex pipiens*	3
Housefly	*Musca domestica*	6
Haploppaus	*Haploppaus gracilis*	2
Garden onion	*Allium cepa*	8
Mustard weed	*Arabidopsis thalliana*	5
Toad	*Bufo americanus*	11
Rice	*Oryza sativa*	12
Adder's Tongue	*Ophioglossum vulgatum*	600
Frog	*Rana pipiens*	13
Alligator	*Alligator mississipiensis*	16
Cat	*Felis domesticus*	19
House mouse	*Mus musculus*	20
Rhesus monkey	*Macaca mulatta*	21
Wheat	*Triticum aestivum*	21
Human	*Homo sapiens*	23
Potato	*Solanum tuberosum*	24
Cattle	*Bos taurus*	30
Donkey	*Equus asinus*	31
Horse	*Equus caballus*	32
Dog	*Canis familiaris*	39
Chicken	*Gallus domesticus*	39
Carp	*Cyprinus carpio*	52

Apart from the diploid species listed above, it is seen that asexually reproducing species have one set of chromosomes, a condition known as Monoploidy [n]. Gametes in diploid species are Haploid [n] and have

one set of chromosomes. These species have somatic cells, body cells, which are diploid [2n] having two sets of chromosomes, one from the mother and one from the father. Gametes are produced by meiosis of a diploid germ line cell.

Some animal and plant species are polyploid [Xn] and have more than two sets of chromosomes. Agriculturally important plants such as tobacco or wheat are often polyploid compared to their ancestral species.

Chromosome Size

The chromosomes of a single genome may differ considerably in size. In the human genome, for example, there is about a three- to fourfold range in size from chromosome 1 (the biggest) to chromosome 21 (the smallest), as shown in Figure 1. In studying the chromosomes of some species, a cytogeneticist may have difficulty identifying individual chromosomes by size alone but may be able to group chromosomes of similar size. A change may then be detected in, for example, "one of the chromosomes in size group A."

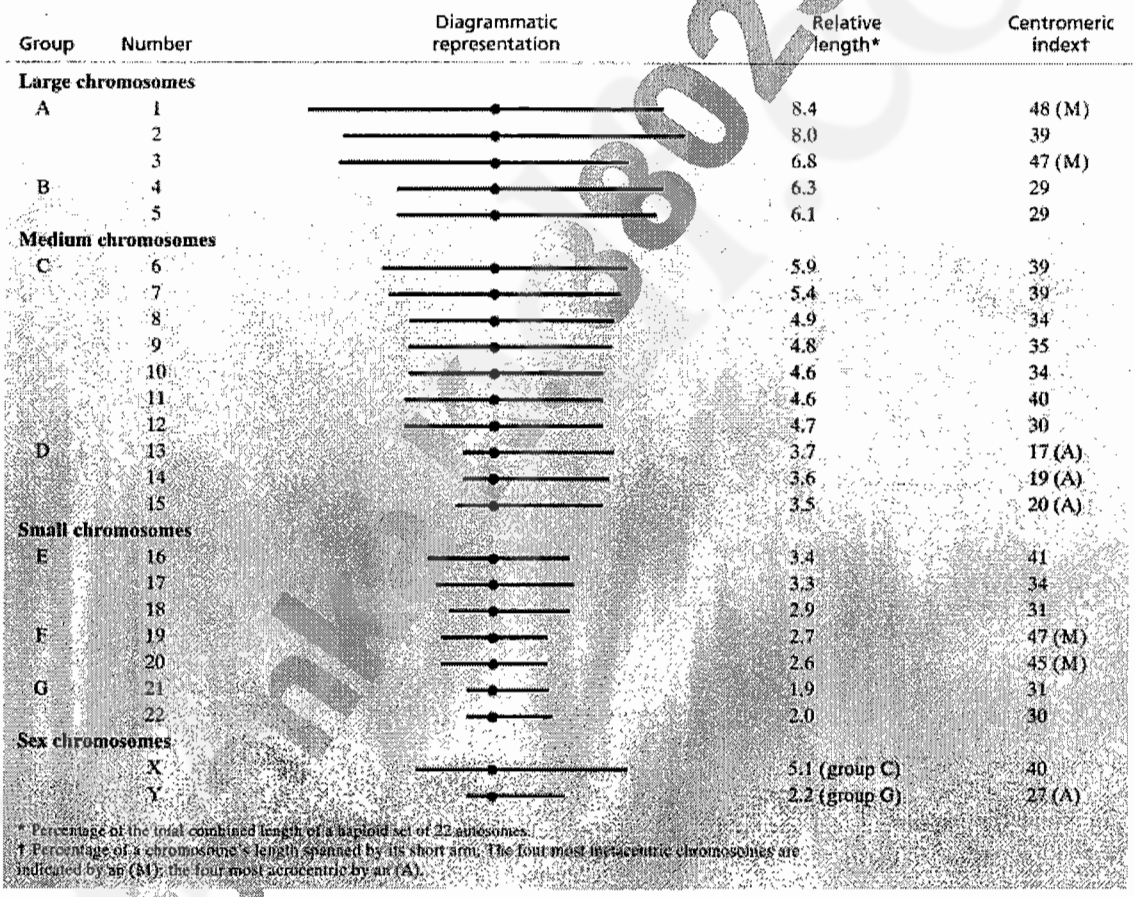


FIGURE 1: Structural Attributes of Human Chromosomes

Centromere Position

The centromere is the region of the chromosome to which spindle fibers attach. The centromere region usually appears to be constricted, and the position of this constriction defines the ratio between the lengths of the two chromosome arms; this ratio is a useful characteristic (Figure 1).

Centromere positions can be categorized as:

1. **telocentric** (at one end),
2. **acrocentric** (off center), or
3. **metacentric** (in the middle).

Note: Cytogeneticists no longer recognize the **sub-metacentric** category, as all the chromosomes having centromere position intermediate between metacentric and telocentric are now recognized as acrocentric. **(Reference: *INTRODUCTION TO GENETIC ANALYSIS*** by Anthony J. F. Griffiths; Susan Wessler; Richard Lewontin; Sean Carroll; Ninth Edition © 2008)

The centromere position determines not only arm ratio, but also the shapes of chromosomes as they migrate to opposite poles during anaphase. These anaphase shapes range from a rod to a J to a V (Figure 2). A telocentric chromosome has its centromere at one end; when the chromosome moves toward one pole of the cell during the anaphase of cellular division, it appears as a simple rod. An acrocentric chromosome has its centromere somewhere between the end and the middle of the chromosome; during anaphase movement, the chromosome appears as a J. A metacentric chromosome has its centromere in the middle and appears as a V during anaphase.

In some organisms, such as the lepidoptera, centromeres are "diffuse," so spindle fibers attach all along the chromosome.

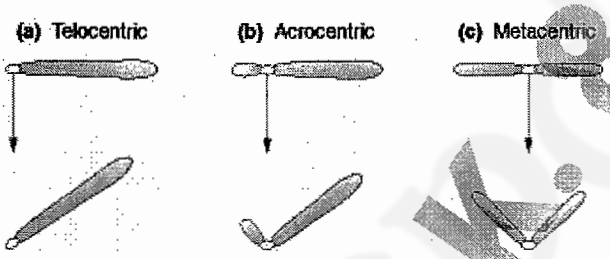


FIGURE 17: The classification of chromosomes by the position of the centromere.

Position of Nucleolar Organizers

Nucleoli are intranuclear organelles that contain ribosomal RNA, an important component of ribosomes. Different organisms are differently endowed with nucleoli, which range in number from one to many per chromosome set. The diploid cells of many species have two nucleoli. The nucleoli reside next to secondary constrictions of the chromosomes, called **nucleolar organizers**, which have highly specific positions in the chromosome set. Nucleolar organizers contain the genes that code for ribosomal RNA. Their positions, like those of centromeres, are landmarks for cytogenetic analysis.

Heterochromatin Patterns and Banding Patterns

When chromosomes are treated with chemicals that react with DNA, such as Feulgen stain, distinct regions with different staining characteristics are visually revealed. Densely staining regions are called heterochromatin; poorly staining regions are said to be euchromatin. The distinction refers to the degree of compactness, or coiling, of the DNA in the chromosome.

There are a number of different staining techniques (Table 2), each resulting in a banding pattern that is characteristic for a particular chromosome. The positions and sizes of the bands are highly chromosome specific. This means that the set of chromosomes possessed by an organism can be represented as a karyogram, in which the banded appearance of each one is depicted.

Table 2. ***STAINING TECHNIQUES USED TO PRODUCE CHROMOSOME BANDING PATTERNS***

TECHNIQUE	PROCEDURE	BANDING PATTERN
G-banding	Mild proteolysis followed by staining with Giemsa	Dark bands are AT-rich Pale bands are GC-rich
R-banding	Heat denaturation followed by staining with Giemsa	Dark bands are GC-rich Pale bands are AT-rich
Q-banding	Stain with quinacrine	Dark bands are AT-rich Pale bands are GC-rich
C-banding	Denature with barium hydroxide and then stain with Giemsa	Dark bands contain constitutive heterochromatin

By using all the available chromosomal landmarks together, cytogeneticists can distinguish each of the chromosomes in many species.

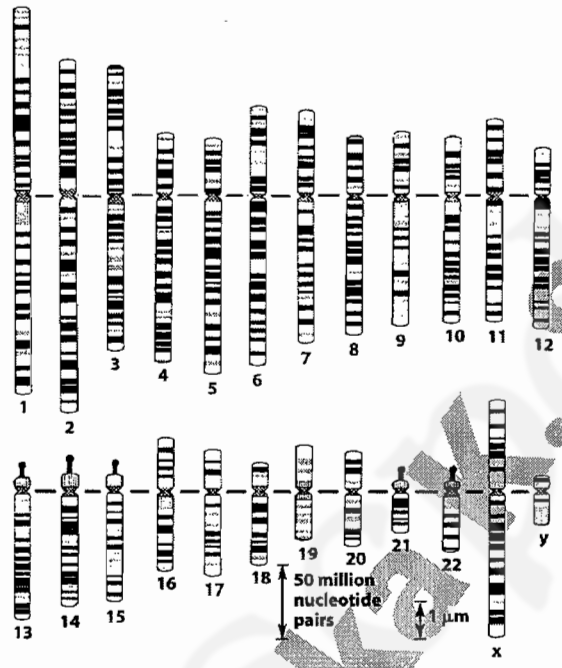


FIGURE 18: The human karyogram. The chromosomes are shown with the G-banding pattern obtained after Giemsa staining. Chromosome numbers are given below each structure.

Molecular anatomy of chromosomes

The length of DNA in the nucleus is far greater than the size of the compartment in which it is contained. To fit into this compartment the DNA has to be condensed in some manner. The degree to which DNA is condensed is expressed as its packing ratio.

(Packing ratio - the length of DNA divided by the length into which it is packaged) .

For example, the shortest human chromosome contains 4.6 x 107 bp of DNA (about 10 times the genome size of E. coli). This is equivalent to 14,000 µm of extended DNA. In its most condensed state during mitosis, the chromosome is about 2 µm long. This gives a packing ratio of 7000 (14,000/2). To achieve the overall packing ratio, DNA is not packaged directly into final structure of chromatin. Instead, it contains several hierarchies of organization (Fig. 4).

1. The first level of packing is achieved by the winding of DNA around a protein core to produce a "bead-like" structure called a **nucleosome**. This gives a packing ratio of about 6. This structure is invariant in both the euchromatin and heterochromatin of all chromosomes.

2. The second level of packing is the coiling of beads in a helical structure called the **30 nm fiber** that is found in both interphase chromatin and mitotic chromosomes. This structure increases the packing ratio to about 40.

3. The final packaging occurs when the fiber is organized in loops, scaffolds and domains that give a final packing ratio of about 1000 in interphase chromosomes and about 8,000 in mitotic chromosomes.

4. Thus to summarize: Eukaryotic chromosomes consist of a DNA-protein complex that is organized in a compact manner which permits the large amount of DNA to be stored in the nucleus of the cell. The subunit designation of the chromosome is chromatin. The fundamental unit of chromatin is the nucleosome.

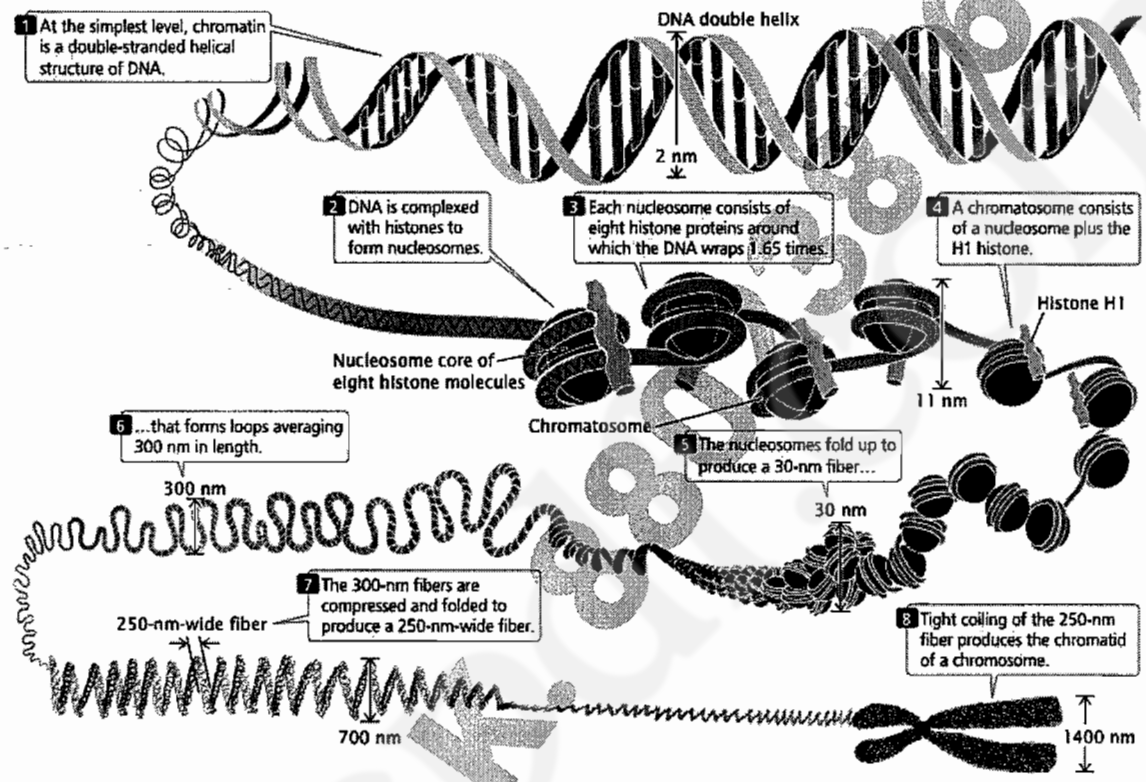


FIGURE 19: The several hierarchies of chromosome organization

Nucleosomes

Nucleosome is the simplest packaging structure of DNA that is found in all eukaryotic chromosomes; DNA is wrapped around a core octamer of small basic proteins called histones; **147 bp of DNA** is wrapped around the core in 1.65 turns and the remaining bases link to the next nucleosome; this structure causes negative supercoiling.

The nucleosomes were discovered and first elaborated by Roger D. Kornberg and coworkers in 1974. When chromatin is isolated from the nucleus of a cell, treated with a very low salt concentration solution and viewed with an electron microscope, it frequently looks like beads on a string. These beads on a string are linear arrays of nucleosomes.

The nucleosome in entirety consists of:

1. about 166 bp DNA wrapped around a histone octamer + single H1 molecule, and
2. linker DNA that is 15 – 105 base pair long which link to the next nucleosome.

The core octamer is composed of two copies of histone proteins H2A, H2B, H3 and H4, which are greatly conserved in various organisms. The core nucleosome has **147 bp of DNA**, wrapped around in 1.65 turns.

The core histones interact with each other in very specific ways. **H3 and H4 form a tetramer** containing two molecules of each $(H3/H4)_2$, while **H2A and H2B form dimers** (H2A-H2B). Under physiologic conditions, these histone oligomers associate to form the **histone octamer** of the composition $(H3/H4)_2$-$(H2A\text{-}H2B)_2$. (Fig. 6)

When the fifth histone H1 (or H5 in birds and reptiles) joins this core, the resulting structure is a chromatosome. A chromatosome has 166 + 2 base pairs of DNA.

Histones are basic proteins that have an affinity for DNA and are the most abundant proteins associated with DNA. They bind with the **minor grooves of the DNA surface** with electrostatic forces (Fig. 5), since the DNA surface is negatively charged due to repeated phosphate groups while the histones are Lysine-Arginine rich positively charged proteins.

The amino acid sequence of the four histones (2A, 2B, 3 and 4) is conserved suggesting a similar function for all.

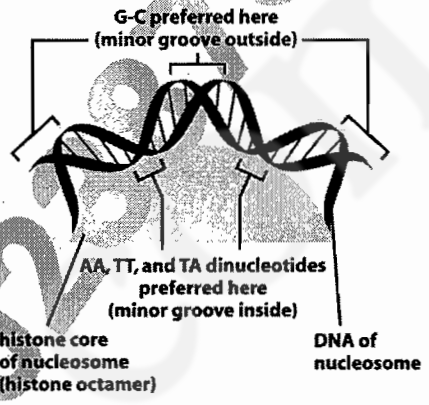


FIGURE 20: Interaction between histone core and DNA

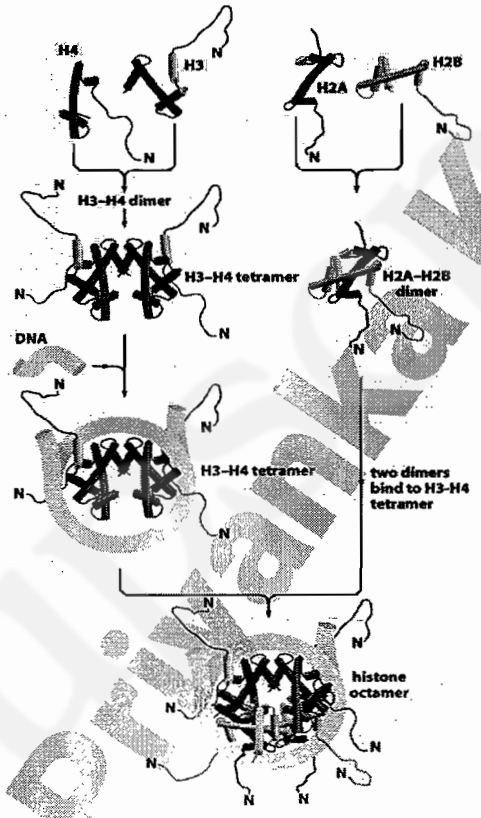


FIGURE 21: The association between Histone Core Proteins and DNA

The length of DNA that is associated with the overall nucleosome unit varies between species, due to linker DNA length variation. But regardless of this, the **Core DNA** (the DNA that is actually associated with the histone octamer) **is invariant and is 147 base pairs**. The DNA that is between two chromatosomes is called the **linker DNA** and can vary in length from 15 to 116 base pairs. This variation is species specific, but variation in linker DNA length has also been associated with the developmental stage of the organism or specific regions of the genome.

About Histones: The most abundant proteins in chromatin are the histones, which are relatively small, positively charged proteins of five major types: H1, H2A, H2B, H3, and H4. Some minor variants of these five major types are found in vertebrates and may replace one of major types in certain tissues or at certain places on the chromosome (for example H5 replaces H1 in birds and reptiles). All histones have a high percentage of arginine and lysine, positively charged amino acids that give the histones a net positive charge. The positive charges attract the negative charges on the phosphates of DNA; this attraction holds the DNA in contact with the histones.

Table 3: *CHARACTERISTICS OF HISTONE PROTEINS*

HISTONE PROTEIN	MOLECULAR WEIGHT	NUMBER OF AMINO ACIDS	PERCENTAGE OF *LYS + ARG*
H1	21,130 Da	223	30.8
H2A	13,960 Da	129	20.2
H2B	13,774 Da	125	22.4
H3	15,273 Da	135	22.9
H4	11,236 Da	102	24.5

Non Histone Proteins: A heterogeneous assortment of nonhistone chromosomal proteins makes up about half of the protein mass of the chromosome. A fundamental problem in the study of these proteins is that the nucleus is full of all sorts of proteins; so, whenever chromatin is isolated from the nucleus, it may be contaminated by nonchromatin proteins. On the other hand, isolation procedures may also remove proteins that are associated with chromatin. In spite of these difficulties, we know that some groups of nonhistone proteins are clearly associated with chromatin.

Nonhistone chromosomal proteins may be broadly into those that serve structural roles and those that take part in genetic processes such as transcription and replication. Chromosomal scaffold proteins are revealed when chromatin is treated with a concentrated salt solution, which removes histones and most other chromosomal proteins, leaving a chromosomal protein "skeleton" to which the DNA is attached.

According to recent evidences, the scaffold during the interphase includes Lamins and Nuclear Matrix Proteins (Fig. 9) while in the metaphase the scaffold is composed of Topoisomerases and Condensin (Fig. 11) (After T. Hirano, Nature Review of Molecular Cell Biology, Vol. 7: 311-322, 2006). These scaffold proteins may help fold and pack the chromosome. Other structural proteins make up the kinetochore, cap the chromosome ends by attaching to telomeres, and constitute the molecular motors that move chromosomes in mitosis and meiosis.

Other types of nonhistone chromosomal proteins have roles in genetic processes. They are components of the replication machinery (DNA polymerases, primases) and proteins that carry out and regulate transcription (RNA polymerases, transcription factors, acetylases). High-mobility-group proteins are small, highly charged proteins that vary in amount and composition, depending on tissue type and stage of the cell cycle. Several of these proteins may be important in altering the packing of chromatin during transcription.

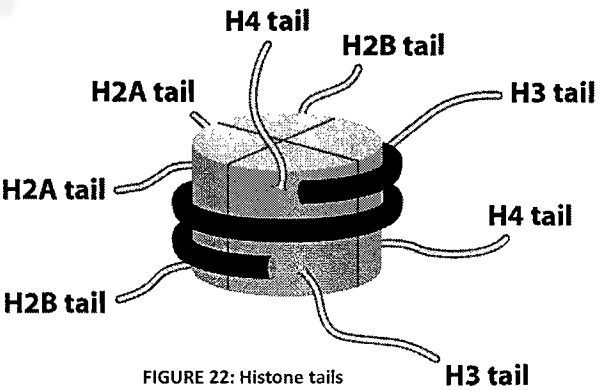


FIGURE 22: Histone tails

Chromatin

Nucleosomes fold on themselves to form a dense, tightly packed structure, called the chromatin structure where adjacent nucleosomes are condensed along the length of the linker DNA. This structure is revealed

when nuclei are gently broken open and their contents are examined with the use of an electron microscope; much of the chromatin that spills out appears as a fiber with a diameter of about 30 nm. It has been proposed that the 10-nm nuleosomal fibril is spontaneously supercoiled with six or seven nucleosomes per turn to form the 30-nm chromatin fiber. In the spontaneous super coiling the positively charged histone tails electrostatically attach to the negatively charged surface of DNA (Fig. 8). Histone tails are positively charged amino acid chains protruding out of the Histone Structure. H2A has two tails, H 1 has no tail – while rest other histones have one tail each (Fig. 7).

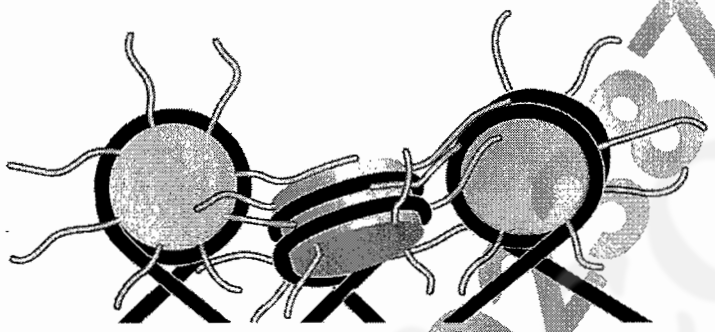


FIGURE 23: Electrostatic Interaction between Histone Tails and Linker DNA

During the interpahse, there is an intranuclear framework, analogous to the cytoskeleton, on which chromosomes and other components of the nucleus are organized. The *nuclear matrix*, or *scaffold*, has been defined as the insoluble material left in the nucleus after a series of biochemical extraction steps.

It has been observed that chromatin folds into a series of looped domains, each containing 20,000–100,000 nucleotide pairs of double-helical DNA condensed into a 30-nm fiber. There appear to be special regions along the looped domains of chromatin called ***SARs* or *MARs* (scaffold-associated or matrix-associated regions)** through which Individual loops can attach to a scaffold formed by nuclear lamina and nuclear matrix proteins (Fig 9). The loops can decondense, when the cell requires direct access to the DNA packaged in these loops. This decondensation is brought about by enzymes that directly modify chromatin structure—as well as by proteins, such as RNA polymerase, that act directly on the underlying DNA. It is not understood how the folded 30-nm fiber is anchored to the chromosome axis, but evidence suggests that the base of chromosomal loops is rich in DNA topoisomerases, which are enzymes that allow DNA to swivel when anchored.

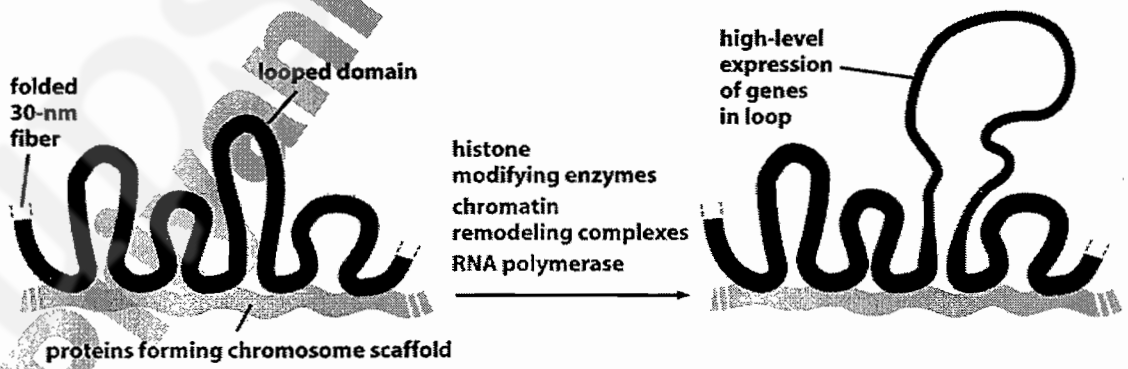


FIGURE 24: A section of an interphase chromosome is shown folded into a series of looped domains along the scaffold formed by nuclear lamina and nuclear matrix proteins during interphase.

Mitotic Condensation

During cell division, the chromosomes become very highly condensed. The DNA in a metaphase chromosome is compacted to about 1/10000 of its stretched-out length.

Figure 10 depicts a typical mitotic chromosome at the metaphase stage of mitosis. The two daughter DNA molecules produced by DNA replication during interphase of the cell-division cycle are separately folded to produce two sister chromosomes, or *sister chromatids*, held together at their centromeres by proteins known as **Cohesins**. In order to allow kinetochore assembly (which is necessary for mitosis) certain histones, especially H3, in the centromeric regions are modified.

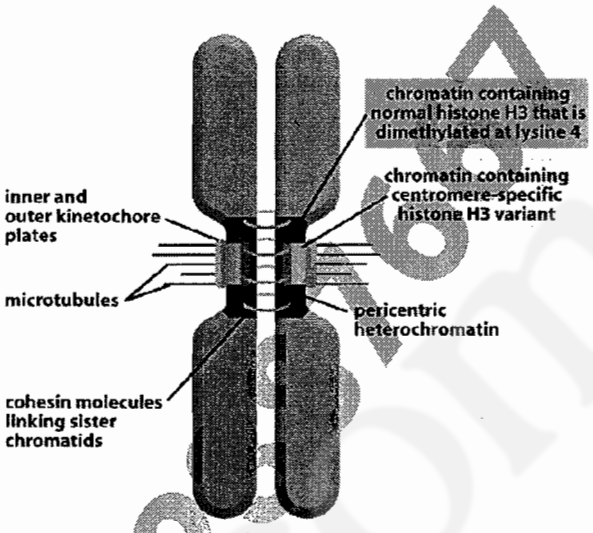


FIGURE 25: A metaphase chromosome

The metaphase chromosomes are normally covered with a variety of molecules, including large amounts of RNA-protein complexes. Once this covering has been stripped away, each chromatid can be seen in electron micrographs to be organized into loops of chromatin emanating from a central scaffolding.

Mitotic chromosome condensation can thus be thought of as the final level in the hierarchy of chromosome packaging . This final level of packaging is characterized by the 1400 nm thick structure seen in the **metaphase chromosome**. Loops of the 30 nm chromatin fiber, containing 20–100 kb of DNA per loop, are attached to a central *scaffold*.

The condensed mitotic chromosome has a characteristic scaffolding structure that can be detected in metaphase chromosomes. This scaffold consists of nonhistone acidic proteins notably **Condensins** and **Topoisomerase II.** In the chromatids of a metaphase chromosome the loop-scaffold complex is compacted yet further by coiling.

It is not yet clear as to how the condensins and Topoisomerase II serve as a central scaffold of a metaphase chromosome. However, the currently accepted model given by T. Hirano (in *Nature Review of Molecular Cell Biology*, Vol. 7: 311-322, 2006) recognizes a central role for *condensins* which use the energy of ATP hydrolysis to drive the coiling of each interphase chromatin that produces a mitotic chromosome. Condensins are large protein complexes that contain SMC proteins: long, dimeric protein molecules hinged in the center, with globular domains at each end that bind DNA and hydrolyze ATP (Figure 11). When added to purified DNA, condensins use the energy of ATP hydrolysis to make large right-handed loops in the DNA. Although it is not yet known how they act on chromatin, the coiling model shown in Figure 11 is based on the fact that condensins are a major structural component of mitotic chromosomes, with one molecule of condensin being present for every 10,000 nucleotides of mitotic DNA.

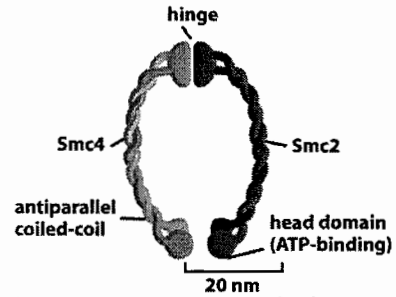


Figure 26A: The structure of condensin

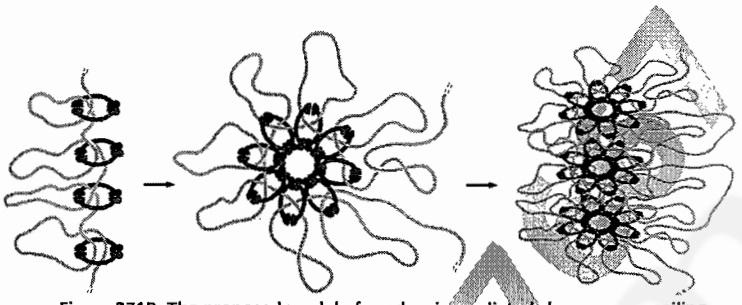


Figure 271B: The proposed model of condensin mediated chromosome coiling.

Additional Information

Functional domains of chromosomes in eukaryotes

The Centromere

Normal chromosomes have a single centromere that is seen under the microscope as the primary constriction, *the region at which sister chromatids are joined*. The centromere is essential for segregation during cell division. Chromosome fragments that lack a centromere (acentric fragments) do not become attached to the spindle, and so fail to be included in the nuclei of either of the daughter cells.

Both the DNA in the centromere regions, and the proteins attached to it, have special features. The nucleotide sequence of centromeric DNA is best understood in the plant *Arabidopsis thaliana*. *Arabidopsis* centromeres span 0.9–1.2 Mb of DNA and each one is made up largely of 180-bp repeat sequences. In humans the equivalent sequences are 171 bp and are called alphoid DNA. Before the *Arabidopsis* sequences were obtained it was thought that these repeat sequences were by far the principal component of centromeric DNA. However, *Arabidopsis* centromeres also contain multiple copies of genome-wide repeats, along with a few genes, the latter at a density of 7–9 per 100 kb compared with 25 genes per 100 kb for the non-centromeric regions of *Arabidopsis* chromosomes (Copenhaver *et al.*, 2001). The discovery that centromere DNA contains genes was a big surprise because it was thought that these regions were genetically inactive.

The special centromeric proteins in humans include at least seven that are not found elsewhere in the chromosome (Warburton, 2001). One of these proteins, CENP-A, is very similar to histone H3 and is thought to replace this histone in the centromeric nucleosomes. It is assumed that the small distinctions between CENP-A and H3 confer special properties on centromeric nucleosomes, but exactly what these properties might be and how they relate to the function of the centromere is not yet known. Part of the function of the centromere itself is revealed by the electron microscope, which shows that in a dividing cell a pair of plate-like kinetochores are present on the surface of the chromosome in the centromeric region. These structures act as the attachment points for the microtubules that radiate from the spindle pole bodies located at the nuclear surface and which draw the divided chromosomes into the daughter nuclei. Part of the kinetochore is made up of alphoid DNA plus CENP-A and other proteins, but its structure has not been described in detail.

During late prophase of mitosis, a pair of kinetochores forms at each centromere, one attached to each sister chromatid. Multiple microtubules attach to each kinetochore, linking the centromere of a chromosome and the two spindle poles. At anaphase, the kinetochore microtubules pull the two sister chromatids toward opposite poles of the spindle. Kinetochores play a central role in this process, by controlling assembly and disassembly of the attached microtubules and, through the presence of motor molecules, by ultimately driving chromosome movement.

Specific DNA sequences presumably specify the structure and function of centromeres. In simple eukaryotes, the sequences that specify centromere function are very short. For example, in the yeast *Saccharomyces cerevisiae* the centromere element (CEN) is about 110 bp long, comprising two highly conserved flanking elements of 9 bp and 11 bp and a central AT-rich segment of about 80–90 bp. The centromeres of such cells are interchangeable - a CEN fragment derived from one yeast chromosome can replace the centromere of another with no apparent consequence. In mammals, centromeres comprise hundreds of kilobases of repetitive DNA, some nonspecific and some chromosome-specific.

Origins of Replication

The DNA in most diploid cells normally replicates only once per cell cycle. The initiation of replication is controlled by *cis*-acting sequences that lie close to the points at which DNA synthesis is initiated. Probably these are sites at which *trans* -acting proteins bind. Eukaryotic origins of replication have been most comprehensively studied in yeast, where the presence of a putative replication origin can be tested by a genetic assay. To test the ability of a random fragment of yeast DNA to promote autonomous replication, it is incorporated into a bacterial plasmid together with a yeast gene that is essential for growth of yeast cells. This construct is used to transform a mutant yeast that lacks the essential gene. The transformed cells can only form colonies if the plasmid can replicate in yeast cells. However, the bacterial replication origin in the plasmid does not function in yeast, therefore the few plasmids that transform at high efficiency must possess a sequence within the inserted yeast fragment that confers the ability to replicate extrachromosomally at high efficiency - that is an autonomously replicating sequence (**ARS**) element.

ARS elements are thought to derive from authentic origins of replication and, in some cases, this has been confirmed by mapping a specific ARS element to a specific chromosomal location and demonstrating that DNA replication is indeed initiated at this location. ARS elements extend for only about 50 bp and consist of an AT-rich region which contains a conserved core consensus and some imperfect copies of this sequence. In addition, the ARS elements contain a binding site for a transcription factor and a multiprotein complex is known to bind to the origin.

Mammalian replication origins have been much less well defined because of the absence of a genetic assay. Some initiation sites have been studied, but such studies have not been able to identify a unique origin of replication. This has led to speculation that replication can be initiated at multiple sites over regions tens of kilobases long. Mammalian artificial chromosomes seem to work without specific ARS sequences being provided. Computer analysis of regions encompassing several eukaryotic origins of replication, including some human and other mammalian examples, identified a consensus DNA sequence WAWTTDDWWWDHWGWHMAWTT where W = A or T; D = A or G or T; H = A or C or T; and M = A or C.

The Telomeres

Telomeres are specialized structures, comprising DNA and protein, which cap the ends of eukaryotic chromosomes. They have several likely functions:

1. Maintaining the structural integrity of a chromosome. If a telomere is lost, the resulting chromosome end is unstable. It has a tendency either to fuse with the ends of other broken chromosomes, to be involved in recombination events or to be degraded. The loop structure of human telomeres means that natural chromosomes have no free DNA end.

2. Ensuring complete replication of the extreme ends of chromosomes. During DNA replication, synthesis of the lagging strand is discontinuous and requires the presence of some DNA ahead of the sequence which is to be copied to serve as the template for an RNA primer. However, at the extreme end of a linear molecule, there can never be such a template, and a different mechanism is required to solve the problem of replicating the ends of a linear DNA molecule.

3. Helping establish the three-dimensional architecture of the nucleus and/or chromosome pairing. Chromosome ends appear to be tethered to the nuclear membrane, suggesting that telomeres help to position chromosomes.

Eukaryotic telomeres consist of a long array of tandem repeats. One DNA strand contains TG-rich sequences and terminates in the 3′ end; the complementary strand is CA-rich. Unlike centromeres, the sequence of telomeres has been highly conserved in evolution - there is considerable similarity in the simple sequence repeat, for example TTGGGG (*Paramecium*), TAGGG (*Trypanosoma*) TTTAGGG (*Arabidopsis*) and TTAGGG (*Homo sapiens*).

The problem of replicating the ends of a chromosome has been solved by extending the synthesis of the leading strand using a specialized enzyme, telomerase. This RNA-protein complex carries within its RNA component a short sequence that will act as a template to prime extended DNA synthesis of telomeric DNA sequences on the leading strand. Further extension of the leading strand provides the necessary template for DNA polymerase α to complete synthesis of the lagging strand. This mechanism leaves the telomere itself with a protruding 3′ end. In mammalian chromosomes, the single-stranded end is believed to loop round and invade the double helix several kilobases proximally, producing a triple-stranded structure resembling the mitochondrial D-loop. However, the actual nature of the telomere sequence may not be important. The telomere length is known to be highly variable and is subject to genetic control.

EUCHROMATIN & HETEROCHROMATIN

Introduction

Chromatin is the complex of genomic DNA and chromosomal proteins present in the eukaryotic nucleus during the interphase. The chromatin structure during the interphase is that of the 30 nm solenoid fiber.

When non-dividing interphase nuclei are properly stained and then examined by light microscopy a mixture of lightly and darkly staining areas within the nucleus can be seen. This differential affinity for the stains is called **heteropycnosis**.

The *dark areas,* or *the positively heteropycnotic parts*, tend to be concentrated around the periphery of the nucleus, and they are called **heterochromatin** and contain DNA in a relatively compact organization, although less compact than in the metaphase structure. Two types of heterochromatin are recognized:

1. **Constitutive heterochromatin** is a permanent feature of all cells and represents DNA that contains almost no genes and so can always be retained in a compact organization. This fraction includes centromeric and telomeric DNA as well as certain regions of some other chromosomes. For example, most of the human Y chromosome is made of constitutive heterochromatin.
2. **Facultative heterochromatin** is not a permanent feature but is seen in some cells some of the time. Facultative heterochromatin contain genes that are inactive in some cells or at some periods of the cell cycle. When these genes are inactive, their DNA regions are compacted into heterochromatin.

On the other hand, the *light areas*, or *the negatively heteropycnotic parts*, tend to be concentrated in the core of the nucleus, and they are called **euchromatin** and contain DNA in a relatively relaxed organization. The euchromatin has greater gene density and most of these genes are transcriptionally active.

It is now well established that this differential packaging of the genetic material during the interphase is a fundamental gene regulation strategy in eukaryotes. The organization of heterochromatin is so compact that proteins involved in gene expression simply cannot access the DNA.

History

The phenomenon of heteropycnosis and the resulting two sectors of the chromatin material in an interphase nucleus (i.e. the euchromatin & the heterochromatin) were first reported by **E. Heitz** in 1928, while working on a liverwort, *Pellia*. A number of later works on many other eukaryotic cells, such as Rat liver cell, gut epithelial cell, lymphocytes, leaf mesophyll cell etc, established that heteropycnosis and the resulting two sectors of the chromatin material in an interphase nucleus are of universal occurrence in eukaryotes.

Cooper (1959) was able to summarize the data from *Drosophila* which suggested that heterochromatin and euchromatin differed in their biophysical conformations and in metabolic expression of their genes but not in their basic structure of DNA arranged within chromosomes.

The recently published report **Finishing the Euchromatic Sequence of the Human Genome** by the International Human Genome Sequencing Consortium (**Nature** 431, 931-945; 21 Oct 2004) highlights the difference in gene density between the euchromatic and heterochromatic sectors of the

genome. This report calls the euchromatin as **Gene Urban Centres** due to a higher gene density and the heterochromatin as the **Gene Deserts** for a sparse gene density.

Distribution & Abundance

It is now well established that heteropycnosis and the resulting two sectors of the chromatin material in an interphase nucleus (i.e. the euchromatin & the heterochromatin) are of universal occurrence in eukaryotes. Within an interphase nucleus, the heterochromatic parts tend to be concentrated around the periphery of the nucleus being attached to the nuclear lamina and lamina associated matrix. The euchromatin on the other hand tends to be clustered in the core of the nucleus.

Based on its distribution, 4 types of heterochromatin are known:

1. **Centromeric Heterochromatin:** Localisd in the centromere
2. **Telomeric Heterochromatin:** Localised in the telomeres
3. **Clustered Heterochromatin:** Organised as large clusters
4. **Intercalary heterochromatin:** Interspersed as patches between the euchromatic parts

In the human genome, the heterochromatin accounts for about 62% of the total chromatin material. In other organisms this proportion is usually less, e.g. in *Drosophila*, about 34% of the genome is heterochromatic, while in *Arabidopsis* about 32% of the genome is heterochromatised.

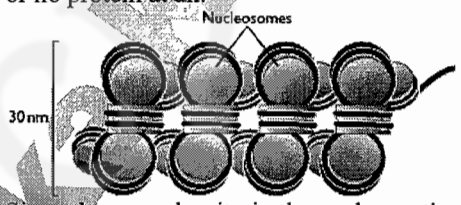
Molecular organization of Euchromatin & Hetero-chromatin

Euchromatin is composed of two types of chromosomal structures:

30-nm fibers, which is the normal relaxed chromatin fiber of the interphase (molecular structure shown in the figure below).

Looped domains, which represent the areas under active transcription – where the DNA is naked (devoid of any structural protein).

In terms of structural packaging, the euchromatin either has Histone proteins or no protein at all.



Since the gene density in the euchromatic parts is high, there are many CpG islands present in these sectors. This makes the euchromatic parts rich in G:C base pairs.

Heterochromatin, in contrast, includes additional proteins and structural RNAs such as XiST and AiST and represents more compact levels of organization. Most DNA that is folded into heterochromatin does not contain genes. As a result we do not find the CpG islands in the heterochromatic. As a result, the heterochromatic parts are rich in A:T base pairs.

Comparison of properties between Euchromatin & Hetero-chromatin

Heterochromatin	Euchromatin
1. Remains condensed throughout interphase (positive heteropycnosis) giving rise to chromocentres.	1. Shows normal cycle of *condensation* during cell division and *extension* during interphase. Does not become *heteropycnotic*.
2. Because of the condensed state, it *stains more heavily* giving rise to banding patterns or chromosomes.	2. Because it is less condensed, it *stains less heavily* (normal staining properties). Only slightly basophilic.
3. Found in *condensed regions* of the chromosome and in association with tight folding or coiling of the chromosomal fibre.	3. Found *diffuse* or *less tightly coiled regions*. Undergoes typical condensation-decondensation cycle.
4. More labile : affected by temperature, sex, age of parents, proximity to the centromere and presence of an additional Y-chromosome.	4. Less *labile* than heterochromatin
5. Does not become *acetylated*.	5. Takes up *acetic acid* (via acetyl CoA) on its histones during interphase.
6. May contain *highly repetitive (satellite) DNA* or *single copy (unique) DNA*.	6. Almost *free of repetitive DNA*. Contains predominantly *single copy DNA*.
7. Relatively *inert* metabolically, but does contain a few genes. The Y-chromosome of *Drosophila melanogaster* and the supernumerary chromosomes of maize are entirely heterochromatin.	7. Genetically *active*.: Almost all the genes are located on euchromatin.
8. DNA is *genetically inert*, and *does not transcribe mRNA* for protein synthesis in the condensed state.	8. *Genetically active*, dispersed part of chromatin in interphase nuclei. Its DNA *synthesizes mRNA* for protein synthesis.
9. *Late replication* of DNA at the S phase of the cell cycle. Under-replication in polytene chromosomes.	9. Comparatively *early replication* of DNA during the early stage of the S phase of cell cycle.
10. *Crossover frequency is less*, because condensed regions of the chromosomal fibre cannot come close together for frequent crossover. This may help protect vital genes from the effects of crossover.	10. *Crossover frequency is more* because of the decondensed (extended) state of euchromatin.
11.Relatively *stable genetically*, with very few mutations mapped.	11. Relatively *less stable*: numerous mutations found.
12. Usually attached to the inside of the *nuclear envelope*.	12. Associated with intranuclear fibrils of the *nuclear matrix*.

The process of Heterochromatin Formation

The process of Heterochromatin Formation is also called **Heterochromatisation**. **The purpose of heterochromatisation is to bring about Gene Silencing** (short **or** long term).

Short Term heterochromatisation is brought about by

1. **Histone deacetylation**
2. **Histone methylation**
3. **Protein binding to the chromatin fibres** such as Polycomb Proteins in *Drosophila* or SiR2 in mammals or HP1 in most of the eukaryotes. (Refer to the description given in the class lecture).

More stable and long term heterochromatisation can be brought about by:

1. **DNA Methylation**
2. **Molecular Caging by structural RNAs** such as XiST (X chromosome inactivation specific transcript) or AiST (Autosome inactivation specific transcript).

It is important to note that heterochromatisation never involves the scaffold proteins which give rise to the metaphase condensation of chromosomes.

Quite often for extensive heterochromatisation, more than one strategies are applied together. X-chromosome inactivation in Human Females for Dosage Compensation is a well known example. In X-chromosome inactivation, the following events take place.

A gene called *Xist*, located in the inactivation center of X chromosome, which is transcribed into a 25-kb non-coding RNA, copies of which coat the chromosome as heterochromatin is formed;

Replacement of histone H2A, one of the members of the core octamer of the nucleosome with a special histone, macroH2A1 (Costanzi and Pehrson, 1998);
Deacetylation of histone H4 (Jeppesen and Turner, 1993), as usually occurs in heterochromatin;
Hypermethylation of certain DNA sequences, although this appears to occur after the inactive state has been set up.

Comparison of properties between facultative and constitutive hetero-chromatin

Constitutive heterochromatin	Facultative heterochromatin
1. *Permanent* part of the genome. Forms the structural characteristic of a chromosome pair.	1. *Temporary* structure. Consists of euchromatin that becomes compact and stainable heterochromatin during *some phase of development.*
2. Not convertible into euchromatin.	2. Undergoes *euchromatization*, i.e. may revert to the euchromatin state.
3. Consists of sequences that are generally found in blocks and concentrated in the same *specific regions* of both homologous chromosomes of a pair. Can be either centromeric, telomeric or intercalary in position. Its possible role is *structural* (e.g. to give centromeric strength) and to act as a *spacer* between vital genes.	3. Takes the form of *entire chromosomes* that are inactive in once cell lineage but can be expressed in others. Examples are the mammalian X-chromosomes and the paternal chromosome set of coccid bugs. Males of mammals have only one X-chromosome. Females have one active *euchromatic* X-chromosome and one that becomes inactive and *heterochromatic* early in development to form the sex chromatin or *Barr body.*
4. *More common type* of heterochromatin, comprising 5-10% of the total chromosomal DNA (upto 40% in *Drosophila virilis*).	4. *Less common type* of heterochromatin, comprising about 2.5% of the total DNA of the genome.
5. Generally the DNA consists of *highly repetitive* DNA sequences (*satellite DNA*), *non-repetitive mainband DNA*, and in the same species, *non-repetitive shoulder DNA*. A high (~68%) *G-C base pair* content gives it a higher buoyant density.	5. The DNA consists of euchromatin that has become compact, and is therefore mostly *single copy* or *unique DNA* which occurs only once in the genome (chromosome set). Single copy DNA consists of a specifically ordered sequence of nucleotides.
6. The genes can replicate, but *do not transcribe mRNA* for protein synthesis. They are therefore *inert* and partly dispensable.	6. Contains *active genes*, but may become condensed and genetically *inactive* under certain developmental and physiological conditions.

Significance of differential chromatin packaging

Controlling genome access is a fundamental strategy of eukaryotic gene expression regulation. Heterochromatisation leads to a compact structure formation that proteins involved in gene expression simply cannot access the DNA. This brings about gene silencing of short term or long term type.
This silencing is critically important for the organism, as shown by the following examples:

1. X- chromosome silencing occurs for the purpose of dosage compensation in mammals.
2. Repression of Flowering Locus C gene by a deacetylase enzyme coded by Flowering Locus D gene in *Arabidopsis* controls the timing of flowering.
3. Cellular differentiation in all the multicellular organisms involves the heterochromatic silencing of many genes which are not going to be expressed for the entire life time of the cell.
4. Apart from silencing the gene, heterochromatisation also:
5. Protects certain parts of the genome from DNA damage or mutagenesis
6. Helps protect the genome from being overtaken by "parasitic" mobile

elements of DNA (Transposons)
7. Enables the proper functioning of telomeres and centromeres
8. Gives rise to the phenomenon called *position effect variegation*, which is responsible for the mottled appearance of the fly eye.

Position effect: Differences in gene expression that depend on the position of the gene on the chromosome and probably reflect differences in the state of the chromatin along the chromosome. The study of position effect variegation has revealed two important characteristics of heterochromatin. First, heterochromatin is dynamic; it can "spread" into a region and later "retract" from it at low but observable frequencies. Second, the state of chromatin—whether heterochromatin or euchromatin—tends to be inherited from a cell to its progeny.

POLYTENE CHROMOSOME

What is a Polytene Chromosome?

Polytene literally means multiple threads, implying that the **Polytene Chromosomes have multiple chromatids attached at a common centromere.** For example, in *Drosophila sp.* the salivary gland cells of the larvae contain the polytene chromosomes which have 512 chromatids lying side by side in each chromosome.

A **polytene chromosome** is a giant chromosomes **found in the interphase nuclei**. It is almost 500 to 1000 times thicker than a normal chromosome and therefore it is visible even with unaided human eyes if properly stained. This is because of polyteny or multistranded nature of these chromosomes.

To increase the cell volume and to meet a short term requirement of high amounts of protein synthesis, some specialised cells undergo **a unique way of gene amplification**, that is to carry out repeated rounds of DNA replication without chromatid separation. This process is called **Endoreduplication** or **Polytenisation**, and it leads to the formation of **Polytene Chromosomes**. Polytenisation is a process of **generalised DNA amplification**. Although most of a chromosome participates in polytenization, certain sequences, such as the simple-sequence DNAs near the centromere, are not amplified.

We should note here that repeated rounds of DNA replication without nuclear division is called **endomitosis** and it leads to polyploidy. Polyteny increases only the C value of the nucleus, while Polyploidisation increases both C and n values.

Occurrence

Polytene Chromosomes were discovered by **E. G. Balbiani** in 1881 in ***Chironomous* larvae.** They routinely occur in the larval salivary glands of many Dipteran insects (such as *Drosophila, Chironomous*, *Bibio*, *Sciara, Rhyncosciara, Camptomya* etc.), antipodal cells of Angiosperm embryosac (as in *Aconitum*), suspensor cells of a young angiosperm embryo, anther tapetal cells of *Vigna unguiculata* and of some *Phaseolus* species and sometimes in the seed endosperm cells. Apart from the insect salivary glands, the cells containing polytene chromosomes are found in the rectal epithelia, gut, footpads and Malpighian Tubules, but the salivary glands are easy to dissect and study.

Cells with polytene chromosomes never divide further, because a proliferation of such a cell would lead to the formation of an aberrant cell line within the organism.

Why Polytenisation occurs?

Polytenisation is a unique method of gene amplification used by certain cells to meet a short term requirement of high amounts of protein synthesis. Such a need generally arises during early development of certain organisms. Having polytene chromosomes enables the organism to cope with this need very effectively. Consequently, most of the cells with polytene chromosomes (such as larval salivary glands, Tapetum, suspensor, and antipodals) have an intense secretory or nutritive role to play. When a gene is present in hundreds or thousands of copies, naturally its product will also be proportionately abundant in the cell.

Moreover, side-by-side alignment of the chromatids ensures that almost all the copies of the gene are expressing together.

Gross Structure

The gross structure of a polytene chromosome is best studied in case of the salivary gland chromosomes of *Drosophila virilis* . The laboratory procedure removes the salivary glands and stains the polytene chromosomes with Feulgen so that they may be observed. *Drosophila virilis* is used instead of *Drosophila melanogaster* because *D. virilis* is much larger and it is easier to dissect and remove the salivary glands from the larvae of this species.

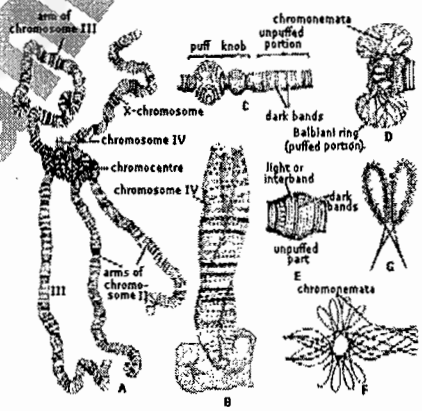
The gross structure is studied with help of light microscopy. A polytene chromosome can be as long as 200 – 600 μm and almost 500 to 1000 times thicker than a normal chromosome.

Appropriately stained Polytene Chromosomes show two remarkable structural features:

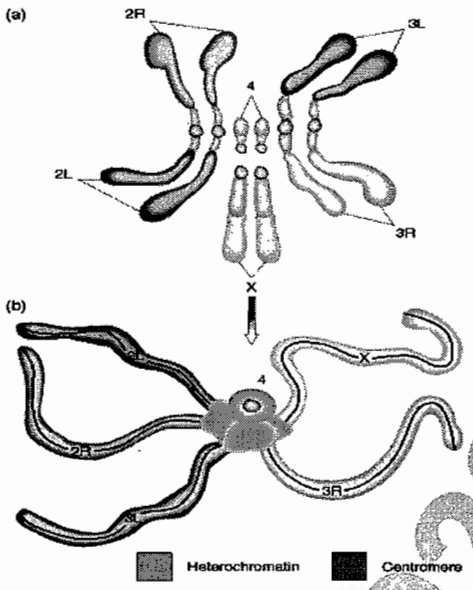
1. Bands and Interbands: Bands are the regions which are strongly Feulgen positive and they form by the juxtaposition of chromomeres of the adjacent chromatids. The interbands are light staining parts amongst the bands. Along the length of a polytene chromosome, there are transverse bands. Polytene bands (about 5000 in total) are much more numerous than Q, G, or R bands, numbering in the hundreds on each chromosome. The bands differ in width and appearance, so that the banding pattern of each chromosome is unique and characteristic of that chromosome.
2. Chromosome Puffs (**Balbiani Rings**) are localized swellings of the chromosome, where the genetic material is in a more relaxed state.

The polytene chromosomes from *Drosophila* also show two additional structural features.

1. The chromosomes despite being in the interphase show homologous pairing, which is also called **Somatic Pairing**.
2. All the four polytene chromosomes become joined at a structure called the ***chromocenter***, which is a coalescence of the heterochromatic areas around the centromeres of all four chromosomes. The chromocenter of *Drosophila* salivary gland chromosomes is shown in the figure below, where L and R stand for arbitrarily assigned left and right arms.



A-C. Salivary gland (polytene) chromosomes of Drosophila melanogaster : A. salivary gland chromosomes; B. chromosome IV enlarged; C. a portion of salivary gland chromosome magnified; D. a chromosomal puff (Balbiani ring) magnified; E. unpuffed portion magnified; F-G. puffing.



Fine structure

The polytene chromosome fine structure can be determined Electron Microscopy, which clearly reveals that each polytene chromosome is composed of many charomatids. Each polytene chromosome usually represents a set of 1024 identical chromatids arranged side by side.

About 95% of the DNA in polytene chromosomes is in bands, and 5% is in interbands. The chromatin in each band appears dark, either because it is much more condensed than the chromatin in the interbands, or because it contains a higher proportion of proteins, or both. Depending on their size, individual bands are estimated to contain 3000–300,000 nucleotide pairs in a chromatin strand. The bands of *Drosophila* polytene chromosomes can be recognized by their different thicknesses and spacings, and each one has been given a number to generate a chromosome "map." There are approximately 5000 bands and 5000 interbands in the complete set of *Drosophila* polytene chromosomes

Under certain conditions (such as high temperature), the bands may exhibit **chromosomal puffs**—localized swellings of the chromosome. Each puff is a region of the chromatin having a relaxed structure and, consequently, a more open state. If radioactively labeled uridine (a precursor to RNA) is briefly added to a *Drosophila* larva, radioactivity accumulates in chromosomal puffs, indicating that they are regions of active transcription. Additionally, the appearance of puffs at particular locations on the chromosome can be stimulated by exposure to hormones such as the insect moulting hormone **Ecdysone** and other compounds that are known to induce the transcription of genes at those locations. This correlation between the occurrence of transcription and the relaxation of chromatin at a puff site indicates that chromatin structure undergoes dynamic change associated with gene activity. Molecular studies have shown that in any chromosomal region of *Drosophila* there are more genes than there are polytene bands, so the bands do not represent genes. **Genes are found in both band and interband regions.**

Some specific proteins bind to polytene chromosomes for Puff Formation; such

as **CHD1** (chromo-ATPase/helicase-DNA-binding domain), and **SNF2/SWI2/Brm** proteins. They are thought to participate in ATP-dependent remodeling (loosening) of chromatin during puffing.

Significance

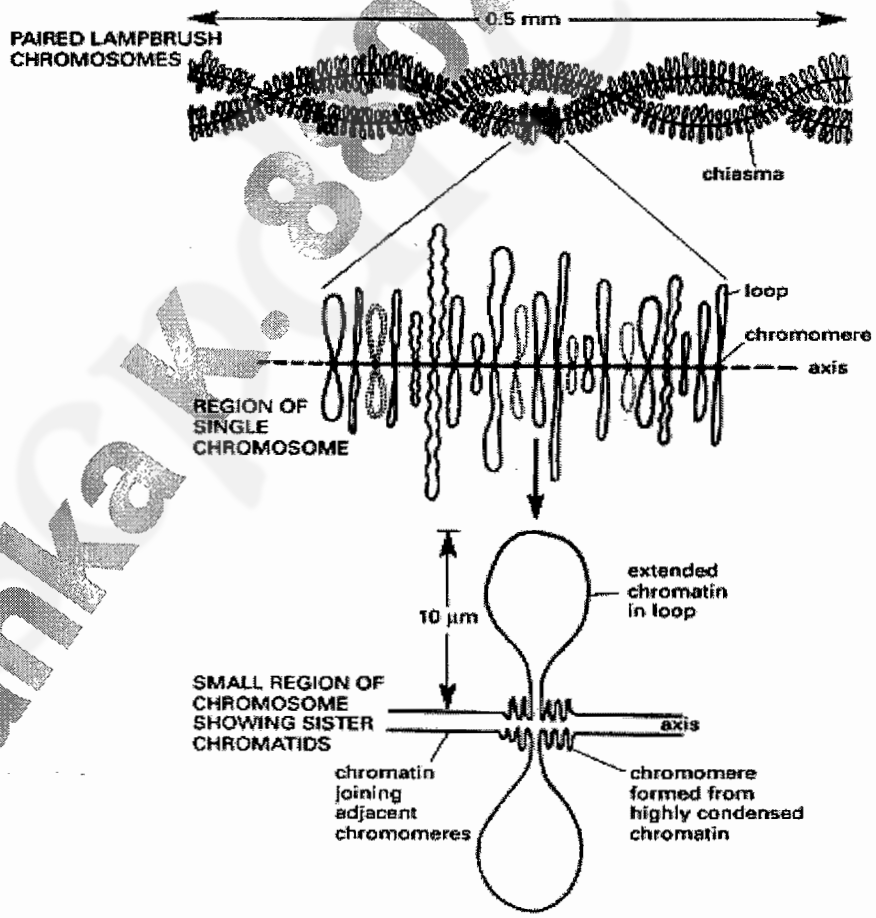
1. In addition to increasing the volume of the cells nuclei and causing cell expansion, polytene cells may also have a metabolic advantage as multiple copies of genes permits a high level of gene expression.
2. The effects of deletion, addition, inversion etc., of chromosomal segments (genes) have been studied in detail due to occurrence of distinct bands and interbands in the polytene chromosomes.
3. Polytene chromosomes show a very consistent pattern of banding and inter-banding. This consistency allows detailed chromosome mapping of the organism.
4. In case of *Drosophila* polyteny, the maternal and paternal homologues remain aligned side by side. This phenomenon is called **somatic pairing**. It allows detection of any physical aberration of the chromosomes such as deletion, inversions and duplications. In such detection exercises, the consistent bands and inter-bands facilitate the identifications. In addition, the pattern of loop formation because of physical chromosomal aberration is an obvious identification tool.
5. Polytene chromosomes are very suitable of in-situ DNA hybridization. The presence of more than 1000 DNA strands in the same molecule allows this excellently.
6. These chromosomes are excellent tools to understand the correlation between chromosome packaging and gene expression. It is one of the earliest systems, where it has been demonstrated that when a gene is expressing, that is transcribing, there must be a localized decondensation of the chromatin material. The puffs [Balbiani Rings] produced in the Polytene Chromosomes have clearly been established as the centres of active transcription. RNA Polymerase Type II has been isolated abundantly from the polytene chromosomes' puffs.
7. With help of these chromosomes, we know that gene transcription and hence overall expression can be induced in eukaryotes alos, just like bacterial cells. When *Drosophila* larvae were treated with Ecdysone, the molting hormone, there was a remarkable increase in the rate of puffing. It shows that a proper stimulus can induce gene expression equally well as it does so in prokaryotes.
8. An obvious correlation between an environmental stimulus and the puffing pattern in the polytene chromosomes has also been used in identifying the location of a particular gene on the chromosome.

LAMPBRUSH CHROMOSOMES

What is a Lampbrush Chromosome ?

A **Lampbrush chromosome** is a giant chromosome, found especially in the maturing oocytes of most of the animals. However, the Lampbrush chromosomes in amphibians is best studied due to an exceptionally large size. LBCs were first seen in sections of salamander (*Ambystoma mexicanum*) oocytes by Flemming in 1882. Ten years later they were described in the oocytes of a dogfish by Ruckert (1892). The name lampbrush comes from Ruckert, who likened the objects to a 19th Century lampbrush, equivalent to the 20th Century test-tube brush.

A Lampbrush chromosome is not a single chromosome. It is actually a homolog pair of chromososmes occuring during the Diplotene of Meiosis Prophase-I. It consists of two chromosomes (hence four chromatids in total) which form many brushlike stiff loops along the main axis of the entire structure. Due to this structure, these giant chromosomes have been called Lampbrush chromosomes.



The above diagram shows that the set of lampbrush chromosomes in most animals contains numerous chromatin loops, although much of the DNA in each chromosome remains highly condensed in the chromomeres. Each loop corresponds to a particular DNA sequence. Four copies of each loop are present in each cell, since each of the two chromosomes shown at the top consists of two closely apposed, newly replicated chromatids. This four-stranded structure is characteristic of this stage of development of the oocyte, the diplotene stage of meiosis.

Development

Lampbrush chromosomes (LBCs) are transitory structures that exist during an extended diplotene of the first meiotic division in females gametocytes of most animals, except mammals. The chromosomes go from a compact telophase form at the end of the last oogonial mitosis, become lampbrushy and then contract again to form normal first meiotic metaphase bivalents. Their most conspicuous feature is widespread RNA transcription from thousands of transcription units that are arranged at short intervals along the lengths of all the chromosomes. In these senses it has been possible to exploit LBCs in the study of chromosome organization and gene expression during meiotic prophase, and in studies of the molecular and supramolecular morphology of RNA transcription.

Structure

A single lampbrush chromosome measures 400-1000 μm in length. They are larger than salivary gland chromosomes. **Being a Meiotic Prophase structure, a Lampbrush chromosome is actually a bivalent of homologous chromosomes. This is revealed clearly by Electron Microscopy. Each homologous chromosome in a Lampbrush Chromosome system is already a replicated structure. Hence, there are four chromatids in a Lampbrush Chromosome. Each homologue makes an axis; thus there are two axes in total.**

A chromatid is always composed of a single DNA molecule, therefore the C value of a Lampbrush Chromosome is 4. Most of the DNA in a given chromatid remains highly condensed in the ***chromomeres*** on the axis. The pairing of both the homologues is extremely precise – chromomere by chromomere. Each chromomere is about 0.25 – 2 μm in diameter. It is sepated from the adjacent chromomere by an average distance of about 1.5 μm.

At many locations, the chromomeres extend out to form large chromatin loops. Since there are 4 chromatids in a **Lampbrush Chromosome system, there would be four lateral DNA loops at any given point – one from each DNA molecule. All the four** loops at a given point always contain the same DNA sequence, and extend in the same manner. Experiments demonstrate that most of the genes present in the DNA loops are being actively expressed. The individual chromatin fibers that make up a loop can be visualized with an electron microscope. Each loop usually contains a single gene. When not expressed, the loop of DNA assumes a thickened structure, possibly a folded 30-nm fiber, but when gene expression is occurring, the loop becomes more extended. RNA Polymerase and nascent RNA have been isolated from the loops of the **Lampbrush Chromosomes, establishing that the loops are the sites of active gene expression.**

It seems likely that the loop structure is made possible by the histone modifying enzymes, chromatin remodeling complexes, and other proteins required for gene expression. They all help to convert the chromomere into a more extended form whenever a gene is expressed.

Functional Role of a Lampbrush Chromosome

In most animals (insects being a major exception), the growing oocyte is active in transcribing genes whose products are (1) necessary for cell metabolism, (2) necessary for oocyte-specific processes, or (3) needed for early development before the zygote-derived nuclei begins to function. These genes are transcribed only in the oocyte and not in any other cell.

The animal oocyte has certain periods of very active RNA synthesis. During the diplotene stage, lamp brush chromosomes provide 4 copies of each gene aligned side by side due to being arrested in Diplotene – providing an opportunity for large scale protein synthesis by coordinated multi-copy gene expression.

Significance

1. Lamp brush chromosomes show a very consistent pattern of chromatin loops. This consistency allows detailed chromosome mapping of the organism.
2. These chromosomes are excellent tools to understand the correlation between chromosome packaging and gene expression. It is one of the earliest systems, in addition to the Polytene chromosomes, where it has been demonstrated that when a gene is expressing, that is transcribing, there must be a localized decondensation of the chromatin material. The loops produced in the Lamp brush Chromosomes have clearly been established as the centres of active transcription. RNA Polymerase Type II has been isolated abundantly from such loops.
3. With help of these chromosomes, we know that the eukaryotes can sustain an increased amount of DNA [4C] if there is a need of increased gene expression.
4. An obvious correlation between an environmental stimulus and the looping pattern in the lamp brush chromosomes has also been used in identifying the location of a particular gene on the chromosome.
5. The Lamp brush chromosomes disprove that gene expression can not take place during cell division.
6. These chromosomes also illustrate how the oocytes accumulate proteins and informosomes (mRNPs) in adequate quantities during their maturation.

B-CHROMOSOMES

Introduction to B-Chromosomes

B-chromosomes are a kind of *supernumerary chromosomes* that may be missing in an organism or may be found as extra chromosomes over and above the standard diploid or polyploid complement of *A-chromosomes* and the sex chromosomes (if any). These are found in natural populations of many plant and animal species. These have been reported in more than 1,000 species of plants belonging to Bryophytes, Pteridophytes, Gymnosperms and Angiosperms.

By definition, these chromosomes are not essential for the life of a species, and are lacking in some (usually most) of the individuals. Thus a population would consist of individuals with 0, 1, 2, 3 (etc) supernumeraries (Burt and Trivers; 2005).

Distribution of B- Chromosomes

B chromosomes have been reported in more than 1000 species of plants (Jones and Rees, 1982) distributed over Bryophytes (including Mosses), Ferns, Gymnosperms and Angiosperms (Both Monocotyledons and Dicotyledons). Mostly, they are found in outbreeders. In the family Poaceae, they are found in 200 species including cereals, and forage grasses belonging to genera *Agrostis. Anthoxonthum. Avena. Bromus, Calamogrostis. Dactylis. Deschompsia, Festuca, Koeleria, Lolium. Pennisetum, Phleum, Secale, Sorghum* and *Zea.*

These B chromosome are often absent in developed agronomic strains, perhaps eliminated due to selection. The B chromosomes may sometimes be restricted only to aerial parts (absent in roots e.g. *Aegilops speltoidas, Ae. mutica)* and may also be eliminated during meiosis. B chromosomes may also increase in number due to a drive involving non-disjunction during pollen mitosis, thus leading to the production of plants with two B chromosomes in the progeny.

The B chromosomes are also found in many insect, lizard and amphibian species.

Properties of B-Chromosomes

1. They are dispensable and are not found in all individuals of a species. They are absent in developed agronomic strains.
2. These may not be formed in all types of cells. Sometimes they are restricted only to aerial parts and absent in roots.
3. These are not homologous with any of the A-chromosomes and during meiosis pair among themselves.
4. Their inheritance is non-Mendelian, sometimes due to *non-disjunction* during pollen mitosis.
5. They are smaller than *A chromosomes* and have their own unique pattern of heterochromatin distribution.

6. Most B chromosomes are mainly or entirely heterochromatic, (and hence they are largely non-coding) but some, such as the B chromosomes of maize, contain significant amount of euchromatic segments.
7. In general, they are genetically inert but may rarely organize nucleoli and carry functional genetic material.
8. When present in high number, they suppress vigor and fertility.
9. They have effect on homologous pairing in species hybrids.
10. They replicate their DNA during late S-phase.
11. The origin and function of these chromosomes are unknown.
12. In plants, there is a tendency for B chromosomes to be present in the germ-line, but to be lost from other tissues such as root tips and leaves.
13. B chromosomes have tendency to accumulate in meiotic cell products resulting in an increase of B number over generations. However, excessive B-chromosome accumulation causes infertility and thus the accumulation effect is counterbalanced by infertility.

Structure of B-Chromosomes

Most B chromosomes are mainly or entirely heterochromatic, (and hence they are largely non-coding) but some, such as the B chromosomes of maize, contain significant amount of euchromatic segments.

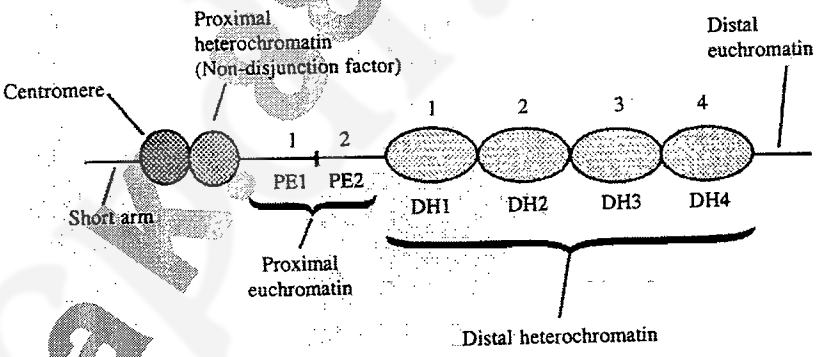


FIGURE 28: B- CHROMOSOME STRUCTURE IN MAIZE

The largest B-chromosome is two-thirds the shortest A-chromosome. Each B-chromosome is composed of short and long arms. Short arm is visible when two B-chromosomes are paired. Long arm is composed of proximal euchromatic region, which is divided into two parts, and distal heterochromatic region is divided into four parts or segments (Fig. 1).

Genes on B-Chromosomes

Usually B-chromosomes are genetically silent. They lack major functional genes. But certain B-chromosomes do exhibit qualitative effects. For example:

1. in *Plantago coronapus,* genes for male sterility and pigmentation.
2. nucleolus organizing region (NOR) as reported in amphibians, insects and plants.
3. genes for achene colour in *Haplopappus gracilis,*
4. gene for leaf-striping in *Zea mays,*

5. gene for meiotic pairing in *Aegilops,*
6. gene for esterase in *Scillia autumnalis*

Formation of *puff* on polytene B-chromosomes of blackfly is interpreted as the site of heavy transcription. Lampbrush B-chromosomes in amphibians also provide evidence of transcriptional activity. Thus, supernumerary chromosomes are not devoid of genetic activity, although they may carry only a few genes.

Effect of B-Chromosomes

There is evidence of deleterious effects of supernumeraries on pollen fertility and favourable effects or associations with particular habitats are also known in a number of species.

The most significant effect of B chromosomes is on pollen fertility and seed set. Flowering time is generally delayed by B chromosomes, and several other characters (plant height, plant weight and tiller number) are adversely affected. There is also an anomalous pattern of variation due to odd and even numbered combinations of B chromosomes. In rye, straw weight and tiller number showed such a difference. Similar effects due to addition of rye B chromosomes to hexaploid wheat have also been shown.

The B chromosomes also have the following effects on A chromosomes:

1. increases asymmetry in chiasma distribution
2. increases crossing over and recombination frequencies: increases variation
3. cause increased unpaired chromosomes: infertility

Evolution of B-Chromosomes

The evolutionary origin of supernumerary chromosomes is obscure, but presumably they must have been derived from heterochromatic segments of normal chromosomes in the remote past.

There is a general opinion that supernumeraries have persisted in a species because there are some positive adaptive advantages due to these chromosomes. In a few cases, it has been identified. For instance, the British grasshopper *Myrmeleotettix maculatus* has two structural types of B chromosomes: metacentric and submetacentric. The B chromosome, which has a satellite DNA, occur in warm, dry environments, and is absent in humid, cooler localities.

CELL CYCLE, PLANT MITOSIS, ITS MOLECULAR BASIS & REGULATION

Cell division is a process of cell replication or cell multiplication in which a pre-existing cell enlarges, replicates its genome, proliferates its membrane component plus the genetically semi-autonomous organelles and the undergoes division to form daughter cells. It is a pre-requisite for growth of multicellular organisms and reproduction (both asexual and sexual).

History of cell division studies

1. Rudolf Virchow (1855, 1859) was the first to suggest that new cells are formed by division of the pre-existing cells – *omnis cellulae cellula* (every cell is derived from a cell).
2. In 1873, Strasburger observed that new nuclei are formed from pre-existing ones.
3. Walter Flemming (1879) studied mitosis for the first time in erythroblasts and epithelial cells of Salamander, *Titurus maculosus*. He found splitting of chromosomes and their equitable distribution in the daughter cells.
4. The term Mitosis was coined by Flemming
5. The double division that occurs prior to the formation of gametes was termed as Meiosis by Farmer and Moore in 1905.
6. The Nobel Prize in Physiology or Medicine for 2001 was awarded jointly to Leland H. Hartwell, R. Timothy (Tim) Hunt and Paul M. Nurse for their discoveries of key regulators of the cell cycle. These scientists through their seminal works in 1970s, 80s and early 90s contributed fundamentally to our understanding of Regulation of the Cell Cycle by Cyclin-CDK Complexes.

The Cell Division Cycle

Cell division cycle consists of three steps (Fig 1):

1. **Interphase** (I-phase): Interphase occupies 75-90% of the total generation time. It prepares the nucleus and the cell to divide.

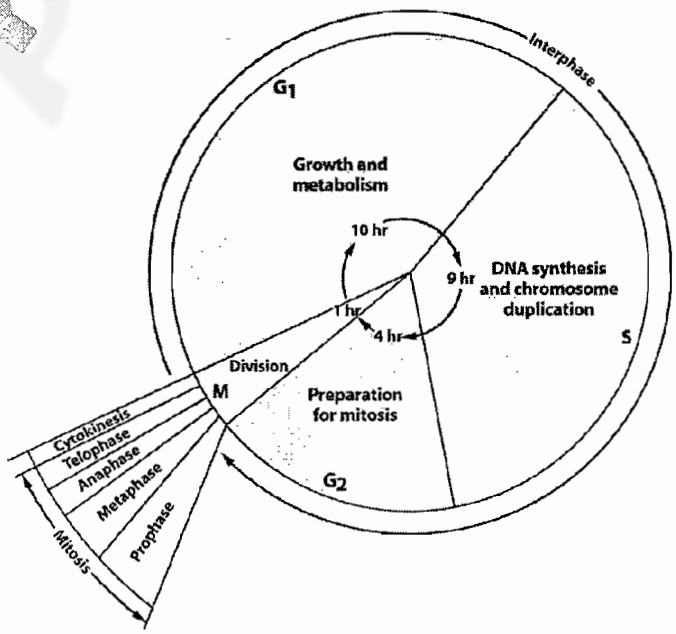


FIGURE 29: STAGES OF THE CELL CYCLE

Interphase is, therefore, also called intermitosis.

2. **Karyokinesis** (M-phase): Karyokinesis (Scheider, 1887) is the division of nucleus.
3. **Cytokinesis** (D-phase) is division of cell protoplast. Some authors include it in the M-phase.

The prokaryotes do not show the mitotic cycle as we find in the eukaryotic cells. Prokaryotes reproduce by cell fission as shown in Figure 2.

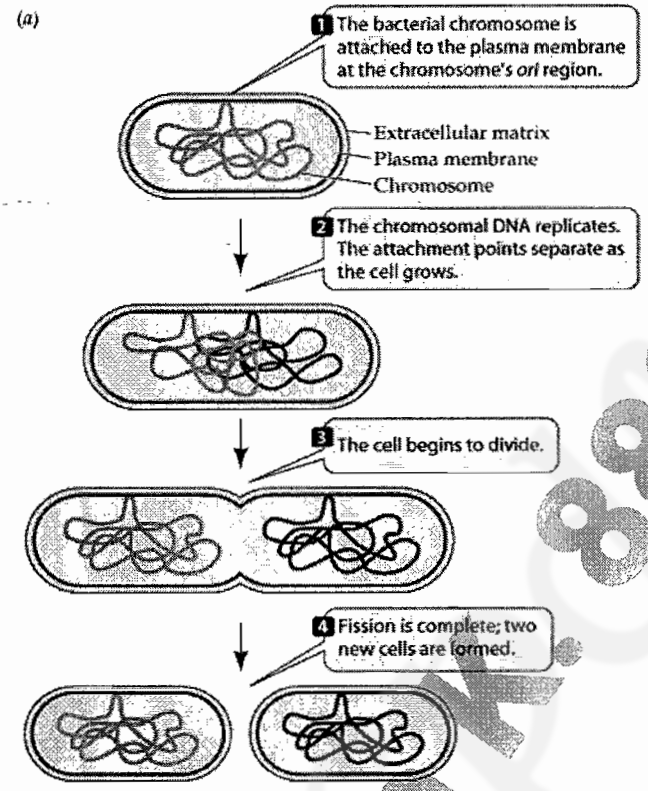


FIGURE 30: BINARY FISSION IN PROKARYOTIC CELLS

Mitotic cell cycle

Cell division is initiated by a mitogenic signal. In plants, this signal is a combination of Auxin and Cytokinin. For root cells, even ethylene acts as a mitogenic signal.

In animals, a number of hormones (*e.g.*, insulin, glucagons, growth hormone, follicle stimulating hormone, triidothyronine, parathormone, progesterone, testosterone) and other factors (*e. g.,* EGF or epidermal growth factor, NGF or nerve growth factor, FGF or fibroblast growth factor, prostaglandins, transferring, serum spreading factor, putreseine, PDGF or platelet derived factor, IL-2 or interleukin 2, IL-3 or interleukin 3, etc.) are known to have mitogenic properties.

Mitogens induce reversible phosphorylation and dephosphorylation of target proteins by specific cell cycle kinases. It starts the cell division cycle.

Interphase

Interphase is a series of changes that take place in a newly formed cell and its nucleus before it develops the ability to divide.

The interphase consists of 3 distinct stages:

1. G_1 : (First Growth Phase, Post-mitotic Gap Phase, Pre-synthetic Phase). It requires two pre-conditions (i) A minimum cell size and (ii) An optimum supply of nutrients. The cell grows in size. Nucleus enlarges slightly. RNAs and proteins are synthesized. A large pool of nucleotides, amino acids and energy rich compounds is formed towards the close of G_1-phase.

   Late in the G1, the decision to divide further is taken by the cell. If the decision is "yes", then growth and preparation of the chromosomes for replication proceeds. If the cell does not have to

divide any further, the cell is said to be arrested in this stage itself, now designated as G_0. This is the most variable stage in terms of its duration.

2. **S** = It is the phase of chromosomal or DNA replication, but only if the cell has decided to divide again. This is the second longest stage in the cell cycle. Histone synthesis also occurs in this period. Euchromatic areas replicate earlier than the heterochromatic areas. At the end of S-phase, each chromatin fibre becomes replicated. Each chromosome comes to have two chromatin fibres which remain attached at a common point called centromere by a protein complex called **Cohesin**. In animal cells, centrosome (centriole pair) replication also occurs in S-phase. *Plant cells do not contain centrosomes.*
3. **G_2** = (Second Growth Phase, Pre-mitotic Gap Phase, Post-synthetic Phase). RNA and protein synthesis continues. A number of macromolecules are formed for multiplication of cell organelles, spindle formation and cell growth. The phase prepares the cell to undergo division.

M-phase of Plant Mitosis

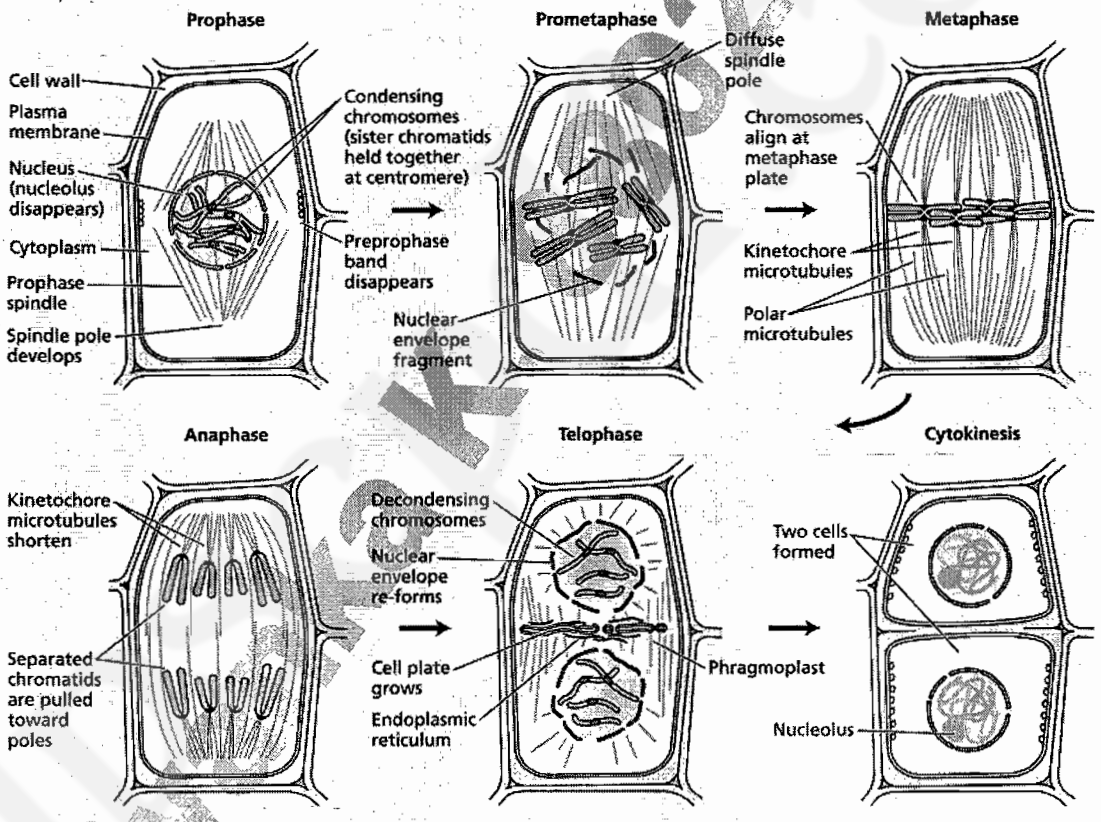


FIGURE 31: THE STEPS IN PLANT MITOTIC M-PHASE

The M-phase of plant mitosis is distinct from animal mitosis. It is summarized in figure 3.

It has the following steps.

1. **Prophase:** It starts with the activation of the M-phase kinase, which is a cyclin CDK complex. It carries out phosphorylation of many targets, such as histones, nuclear matrix proteins, microtubular organizing centre, nucleolar proteins etc.

   As a result of these phosphorylations, in early prophase the chromatin fibres shorten and thicken to form elongated chromosomes. During condensation, chromatin loops are brought together into a compact state due to action of chromosome scaffold proteins. Chromosomal condensation proceeds through the prophase and the **sister chromatids** become visible. The other developments are as follows.

   - The nucleolus begins to disperse.
   - The nuclear envelope begin to disintegrate.
   - The cytoskeletal elements get phosphorylated and start getting reorganized. At this point, the *pre-prophase band of microtubules also disappear.*
   - The *microtubule organizing centres* migrate to their respective poles and begin to form the spindle fibres. The spindle apparatus of plant cells is called **anastral** (Gk, *an-* not *aster*-star) because they do not possess asters.

2. **Prometaphase:** At the end of prophase, the chromosomes are found to lie scattered throughout the space of degenerated nucleus or nuclear region. Spindle apparatus is partially organised with fibres running over or around the nuclear region. Soon some discontinuous spindle fibres get connected with chromosomes in the region of their chromochores. They are called kinetochore fibres.

   Each chromosome gets connected to both the spindle poles by separate fibres. The chromosomal fibres tighten and bring the chromosomes on the equator of the spindle. The bringing of the chromosomes on the equator of the spindle is termed as **congression.** Some condensation of the chromosomes also occurs during the prometaphase.

3. **Metaphase:** The chromosomes are the shortest and the thickest in metaphase. They get arranged on the equatorial plane of the spindle with smaller ones towards the interior and larger ones towards the periphery. The centromeres of all the chromosomes form an apparent plate called **metaphasic plate** or equatorial plate. Each chromosome of the metaphasic plate is connected to both the spindle poles by separte fibres called chromosomal fibres or tractile fibrils. The connection is in the region of centromere which is covered for this by two distinct **kinetochores** on the two surfaces facing the two poles. The balanced tension exerted by the two fibres

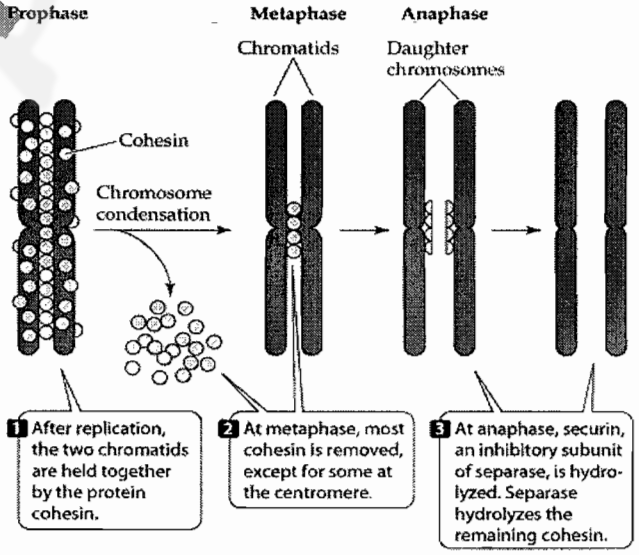


FIGURE 32: SEPARATION OF CHROMATIDS IN ANAPHASE

attached to the centromere of a chromosome and the two poles helps the chromosomes to maintain themselves on the equatorial plane.

4. **Anaphase:** The **Cohesin** holds sister chromatids together from the time they are formed in DNA replication until the onset of anaphase. **Separin hydrolyzes cohesin when its inhibitory subunit, securin, is hydrolyzed by a Ubiquitin Ligase Complex called – APC** (Anaphase Promoting Complex).
This splits each chromosomes into two. All the chromosomes of a metaphasic plate split almost simultaneously.

   The two daughter or new chromosomes formed from a chromosome are attached to the two different poles of the spindle by their own fibres. They move towards the poles of spindle along the path of their spindle fibres.

   Poleward movement of chromosomes by mainly spindle shortening in plants. In animals, it is also by the movement of the spindle poles farther apart.

   At the end of anaphase, two groups of chromosomes are formed, one at each pole of the spindle. The chromosomal fibres disappear. However, the spindle apparatus persists for some time.

5. **Telophase:** It is the reverse of prophase. The two chromosome groups formed at the end of anaphase reorganize themselves into nuclei. The chromosomes elongate and form chromatin fibres. The nucleolar chromosomes produce nucleoli from **prenucleolar bodies** (PNBs). It is is called **nucleologenesis.**

   Nucleoplasm collects in the area of chromatin.

   A nuclear envelope appears on the outside from pieces of the older nuclear envelope and endoplasmic reticulum. In this way two daughter nuclei are formed at the poles of spindle apparatus.

   Meanwhile, the spindle fibres disappear around the poles.

Plant Cytokinesis

It is occurs by two methods, cleavage and cell plate.

1. **Cleavage Method.** It takes place usually in some lower plants. Cytoplasm undergoes centripetal constriction in the middle to form two daughter protoplasts, each having a single nucleus. In the furrow, between the two protoplasts, pectin pectin hemicellulose and cellulose microfibrils are deposited to form a double wall. Wall deposition is generally centripetal like the cytoplasmic constriction.

2. **Cell Plate Formation during Plant Cell Mitosis:** During Mitosis in higher plants, cytokinesis begins with the formation of the cell plate. This process involves fusion of many small secretory vesicles, and attachment of the resulting structure to the plasma membrane. A diagram of the steps involved in cell plate formation is shown below:

   1. In the first step, Golgi vesicles, some of which are interconnected via *fusion tubes,* aggregate in the spindle midzone area. This structure is called the fusion tube network (FTN).
   2. Formation of a tubulo-vesicular network (TVN). The contents of the vesicles, mainly pectins, represent the precursors from which the new middle lamella is assembled outside the cell.

3. In the next stage, vesicle fusion increases, forming a tubulo-vesicular network (TVN), and the membranes become coated with either clathrin or other proteins.
4. After this, the central region of the growing cell plate forms a tubular network (TN), with vesicle fusion occurring at the growing edges where the remaining microtubules are located.
5. In the final stage, the cell plate contacts and adheres to the plasma membrane of the parent cell. At the same time the tubular network expands to form a fenestrated sheet.
6. At the end of mitosis, the phragmoplast disappears, the cell enters interphase, and microtubules reappear in the cytosol near the plasma membrane, where they play a role in the deposition of cellulose microfibrils during cell wall growth.

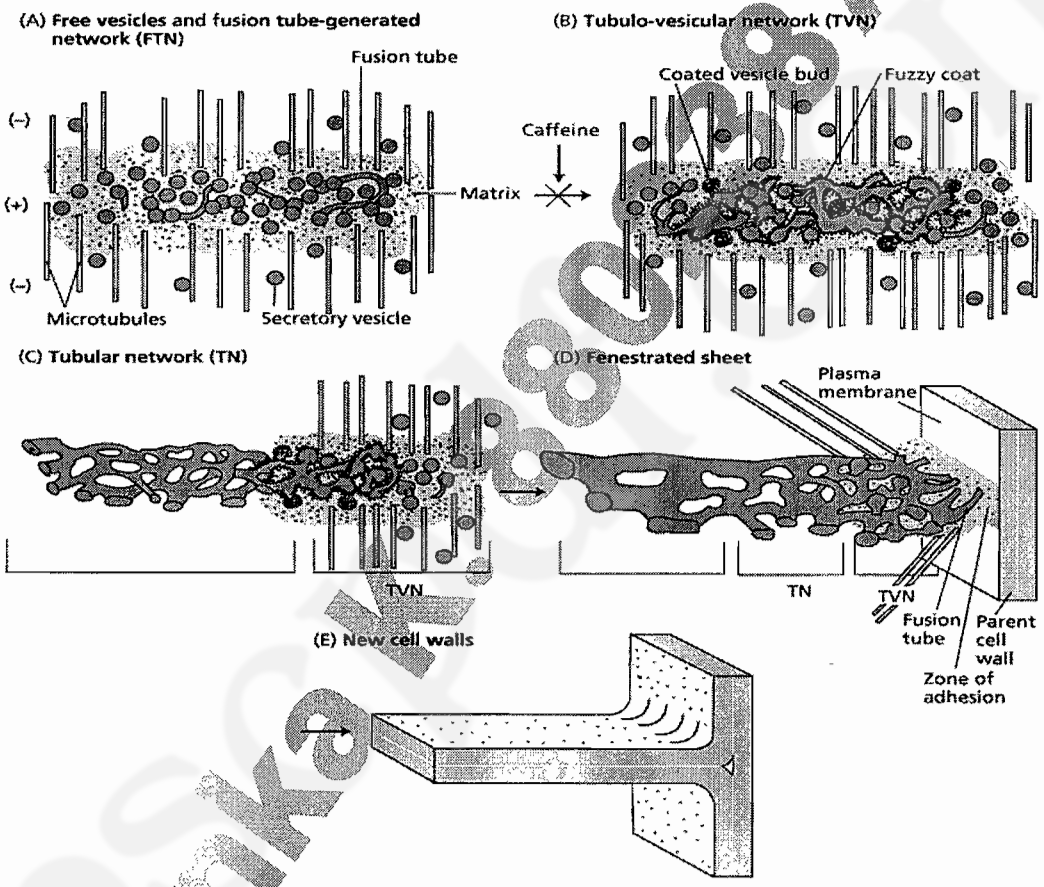


FIGURE 33: PLANT CYTOKINESIS

Significance of Mitosis

1. **Growth and Development.** Somatic cells are formed by mitosis. Therefore, mitosis is essential for growth and development of a multicellular organism.
2. **Cell Size.** It helps in maintaining surface volume ratio or cell size by inducing the overgrown cell to divide.
3. **Karyoplasmic or Nucleocytoplasmic Ratio.** Mitosis maintains the karyoplasmic or nucleocytoplasmic ratio fro efficient control of cellular activities.

4. **Maintenance of Chromosome Number.** Mitosis is accompanied by replication and equitable distribution of all the chromosomes so that all the cells come to have the same number and type of chromosomes. This helps in proper coordination among different cells.
5. **Genetic Similarity and Regeneration.** Mitosis keeps all the somatic cells of an organism genetically similar, resembling the fertilized egg. They, therefore, are able to regenerate part or whole of the organism.
6. **Asexual Multiplication.** Mitosis is involved in asexual reproduction and regeneration.
7. **Multiplication of Meiocytes.** It increases the number of meiocytes inside gonads and sporangia.
8. **Replacement.** It is a mechanism for replacing old or worn out cells. The number of the latter is very high in animals. For examples, in an average human being about 500 billion cells are lost daily.
9. **Healing.** An injury or wound is healed by repeated mitosis divisions of the surrounding healthy cells.
10. **Somatic Mutations.** Mitosis maintains the useful somatic mutations as it is involved in vegetative propagation of plants, *e.g.,* Novel Orange.

Cell Cycle Regulation

The fundamental purpose of any cell division, viz. duplication of the genome and its equal distribution between the two daughter cells can be achieved only when the events of the cell cycle proceed in the correct order. It means that:

1. The DNA replication must always alternate with Mitotic division. In other words, before every cell division, the DNA of the cell must replicate and when DNA replication has taken place, the cell must undergo a division.
2. The chromosome cycle is coordinated with the protoplasmic growth, so that there is no progressive loss or gain of protoplasm through successive rounds of cell divisions.
3. All the events of the cell cycle are operating in a strictly linear manner.
4. All the events of the cell cycle are operating in a dependent manner, which means that a given process must start only after a successful completion the previous step.
5. No step of the cell cycle is repeated.
6. The cell cycle halts if there is any persistent damage to the genetic material.

The cell cycle is a highly regulated molecular process to ensure that all the above requisites are met accurately in each cell cycle.

In case of any inaccuracy, the progression of the cell cycle is halted by the **cell cycle checkpoints**.

There are 4 **major check points**.

1. **Towards the end of the G-1 phase**, where **chromosome replication** is decided. This is also known as **Commitment Point** or **G1 / S checkpoint**. In yeast cells, it is called **START** and in animal cells, it is known as **Restriction Point**. In most of the diploid organisms, this is the major checkpoint, which means that if a cell passes through the restriction point, it would definitely go through the division cycle.
2. **Checkpoint during DNA Replication** which ensures DNA integrity.
3. **At the end of G-2 phase** when **commitment to mitosis** is finalized.
4. **The M phase checkpoint** ensures the precise alignment of the chromosomes on the spindle apparatus, so that the genetic material is divided between the daughter cell equally.

The mechanism of regulation

The fundamental strategy of cell cycle regulation is conserved throughout the eukaryotes.

Modulation of activity by phosphorylation (catalyzed by kinases) and deactivation by de-phosphorylation (catalyzed by phosphatases) of key proteins mediate the various events of the cell cycle.

The complex that brings about periodic phosphorylation of key proteins of cell cycle events is a cyclin-kinase holoenzyme. *The kinase sub-unit is catalytic in nature, while the cyclin molecule binds reversibly to the kinase part and gives rise to the necessary conformational change to stimulate kinase activity. For this reason, the cell cycle kinases are known as* cyclin dependent kinases [or CDKs].

Both kinases and cyclins are highly diverse groups. A table below summarizes their diversity.

In yeast cells, a single Cdk protein (**Cdc2** in *Schizosaccharomyces pombe* and **Cdc28** in *Saccharomyces cerevisiae*) *binds all classes of cyclins and drives all cell-cycle events by changing cyclin partners at different stages of the cycle.*

Organism/Protein	Name
S. POMBE	
CDK (one only)	Cdc2
Mitotic cyclin (one only)	Cdc13
S. CEREVISIAE	
CDK (one only)	Cdc28
Mid G_1 cyclin	Cln3
Late G_1 cyclins	Cln1, Cln2
Early S-phase cyclins	Clb5, Clb6
Late S-phase and early mitotic cyclins	Clb3, Clb4
Late mitotic cyclins	Clb1, Clb2

The basic events of yeast cell cycle regulation are as follows (Fig. 6).

1. DNA replication is the first irreversible commitment to cell division. This decision is taken during the G1 in response to yeast mating factor signaling.
2. In early S-phase, DNA pre-replicative complex (Pre-RC) gets activated by S Phase CDK-Cyclin complex. Now, DNA replication starts.
3. During S-phase itself, the M-phase cyclins are synthesized but they are inhibited by phosphorylation at a pair of amino acids in the roof of the active site. Phosphorylation of these sites is carried out by protein kinases known as **Wee1** and **Myt 1**.
4. After the completion of DNA replication, the cell is in G2. The G_2 cell has two accurate copies of the entire genome. Each replicated chromosome consists of two identical *sister chromatids* attached by **cohesins**.
5. After successful DNA replication, the M-phase cyclin CDK complex is activated by removal of repressory phosphorylations which took place in S-phase by action of protein kinases known as **Wee1** and **Myt 1**. The dephosphorylation is caused by a phosphatase known as **Cdc25.** After this, the **M-Cdk is active**.
6. **M-Cdk**, once activated triggers chromosome condensation by Histone Phosphorylation, nuclear envelope breakdown, actin cytoskeleton rearrangement, and the reorganization of the Golgi apparatus and endoplasmic reticulum. Each of these events is thought to be triggered by M-Cdk phosphorylating specific structural or regulatory proteins involved in the event.

7. When the mitotic spindle attaches to the chromosome, it releases **Mad proteins** from the kinetochore regions. This causes the activation of the Anaphase Promoting Complex (APC).
8. The APC is a Ubiquitin Ligase system causing protein destruction. It has two main targets.
    a. The protein **securin**, which represses the separase enzyme. The destruction of securin at the end of metaphase releases separase, which is then free to cleave one of the subunits of the cohesin complex.
    b. The CyclinB protein. Its destruction inactivates the M-phase cyclin. (i.e. CyclinB is marked for Degradation).
9. After the chromosomes have been segregated to the poles of the spindle, the cell must reverse the complex changes of early mitosis. The spindle must be disassembled, the chromosomes decondensed, and the nuclear envelope reformed. Because the phosphorylation of various proteins is responsible for getting cells into mitosis in the first place, the dephosphorylation of these same proteins is required to get them out. These dephosphorylations and the exit from mitosis could be triggered by the inactivation of M-Cdk, the activation of phosphatases, or both. Evidence suggests that M-Cdk inactivation, plus a telophase specific Phosphatase activation are primarily responsible.
10. The daughter cells, after cytokinesis, are released into G1.

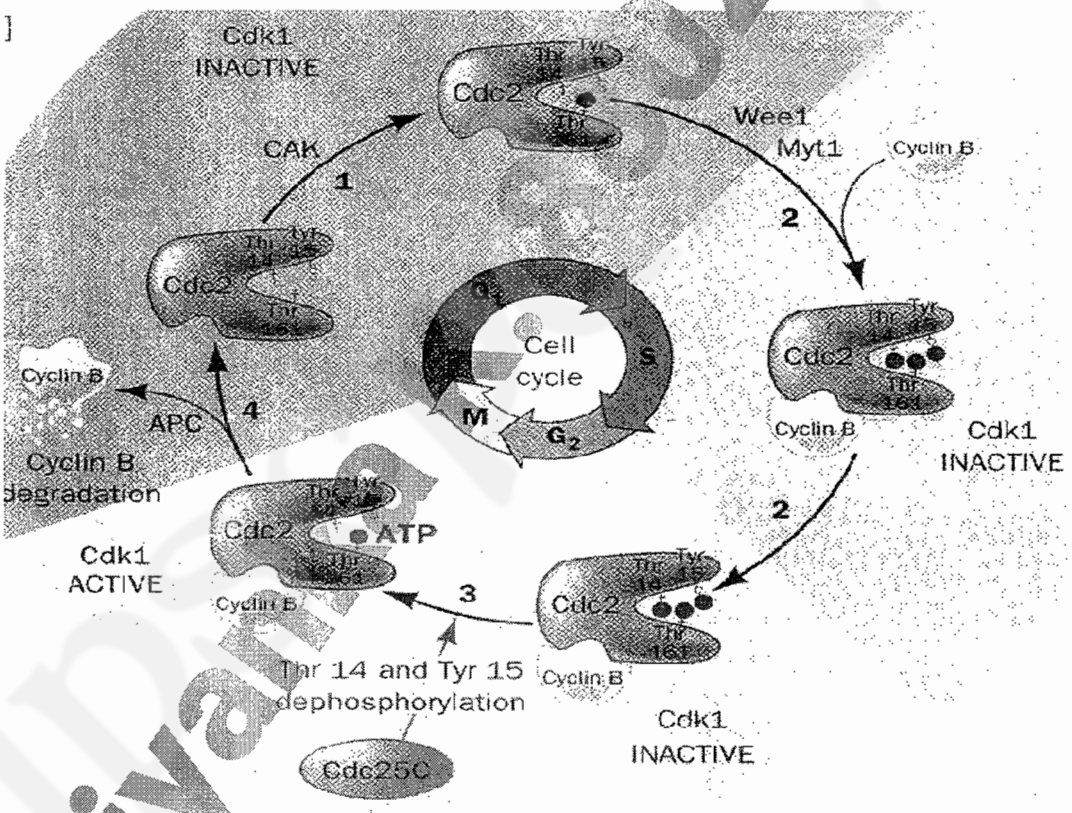


FIGURE 34: MAIN STEPS IN YEAST CELL CYCLE REGULATION

MEIOSIS

Introduction

Meiosis (a Greek word meaning "decrease") is a cellular process occurring strictly in sexually reproducing eukaryotes that forms the basis for sexual reproduction, together with syngamy. It is a form of nuclear division by which a diploid parent, with a duplicated genome (hence the DNA content is 4C) produces four haploid daughter cells. The process includes the two stages of nuclear division (Meiosis I and II), and each is usually accompanied by cell division. In 14 July 2005 issue of *Nature* [Volume 436, Number 7048], single-stage Meiosis has been reported to take place in germ cell development of the fish *Hoplosternum* but not convincingly demonstrated in other organisms.

In diploid organisms, this type of cell division takes place in gonads during gametogenesis only. It consists of two successive divisions called first meiotic and second meiotic divisions. It is during the interphase of first meiotic division that the DNA is replicated in the usual manner leading to Duplicated Diploid amount of DNA in the nucleus and during first meiotic division only the amount of DNA is reduced to Duplicated Haploid since the chromosome number is halved to haploid. In Meiosis-II the DNA in each new daughter cell is reduced to pure haploid by division of the chromatids in the duplicated haploid nucleus.

The Law of DNA Constancy, especially its Transgenerational Fidelity aspect demands that each sexually reproducing organism must undergo meiosis atleast once in its life history.

In higher eukaryotes sexual reproduction involves:

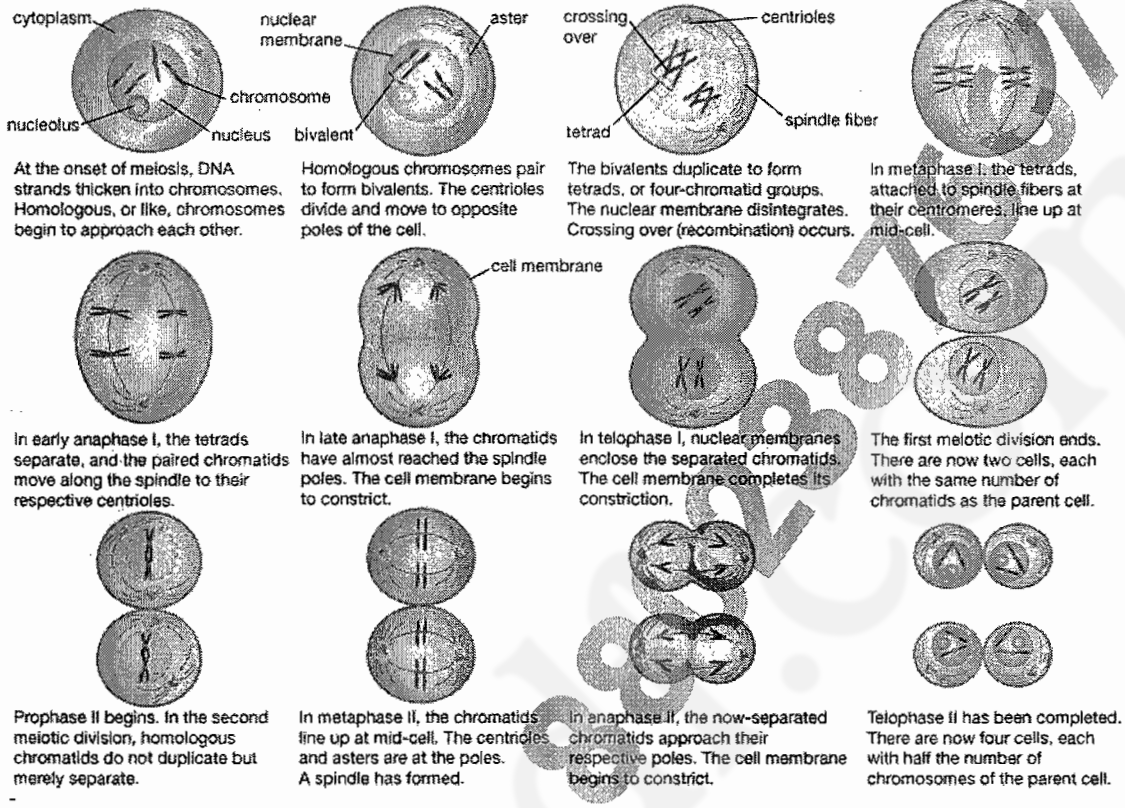
- Formation of new individual by a combination of two haploid sex cells (gametes).
- Fertilization- combination of genetic information from two separate cells that have one half the original genetic information
- Gametes for fertilization usually come from separate parents
    1. Female- produces an egg
    2. Male produces sperm
- Both gametes are haploid, with a single set of chromosomes
- The new individual is called a zygote, with two sets of chromosomes (diploid).

Meiosis is a process to convert a diploid cell to a haploid gamete, and cause a change in the genetic information to increase diversity in the offspring.

Both first and second meiotic divisions can be further subdivided into the same four stages-

1. Prophase
2. Metaphase
3. Anaphase
4. Telophase

Meiosis, or sex cell division



At the onset of meiosis, DNA strands thicken into chromosomes. Homologous, or like, chromosomes begin to approach each other.

Homologous chromosomes pair to form bivalents. The centrioles divide and move to opposite poles of the cell.

The bivalents duplicate to form tetrads, or four-chromatid groups. The nuclear membrane disintegrates. Crossing over (recombination) occurs.

In metaphase I, the tetrads, attached to spindle fibers at their centromeres, line up at mid-cell.

In early anaphase I, the tetrads separate, and the paired chromatids move along the spindle to their respective centrioles.

In late anaphase I, the chromatids have almost reached the spindle poles. The cell membrane begins to constrict.

In telophase I, nuclear membranes enclose the separated chromatids. The cell membrane completes its constriction.

The first meiotic division ends. There are now two cells, each with the same number of chromatids as the parent cell.

Prophase II begins. In the second meiotic division, homologous chromatids do not duplicate but merely separate.

In metaphase II, the chromatids line up at mid-cell. The centrioles and asters are at the poles. A spindle has formed.

In anaphase II, the now-separated chromatids approach their respective poles. The cell membrane begins to constrict.

Telophase II has been completed. There are now four cells, each with half the number of chromosomes of the parent cell.

First Meiotic Division

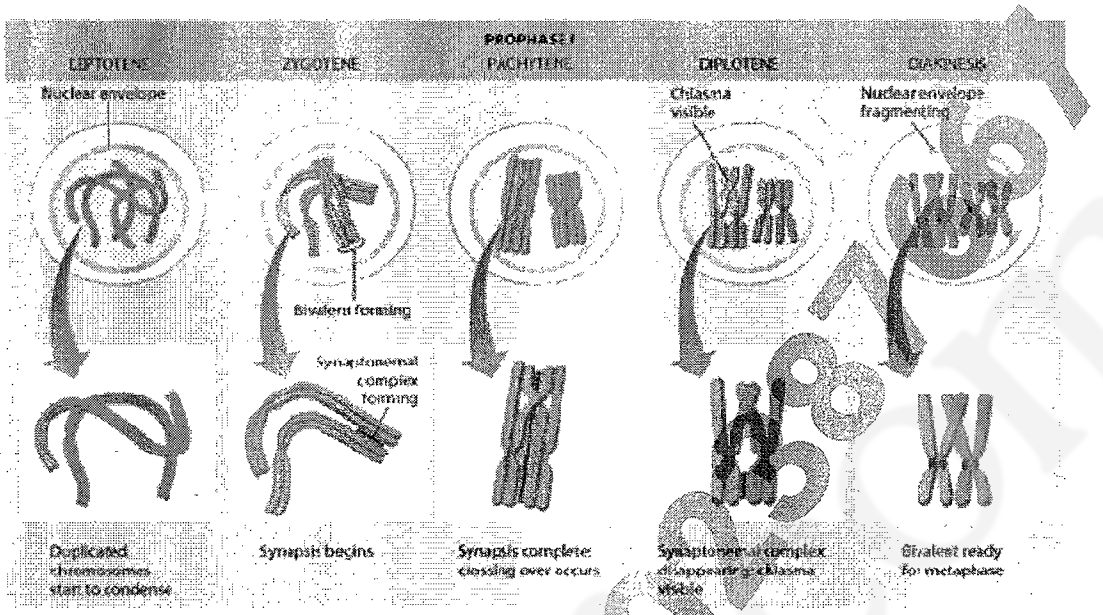
Prophase 1

Prophase I (the prophase of meiosis I) is the longest phase. In this phase, chromosomes shorten and become visible as single threadlike structures. Beaded appearances, if any, are due to the alternation of densely stained chromomeres in comparison with non-staining areas. Chromomeres are regions where the chromosomal material is tightly coiled.

Homologous chromosomes derived from maternal and paternal gamete nuclei come together and pair up. They have same lengths, same centromere positions and in most cases same number of genes arranged in linear order. The pairing process, known as synapsis, may begin at several points along the structures. The paired homologous chromosomes, now known as bivalents, shorten and thicken by means of molecular packaging as well as coiling. The bivalents are now very much visible.

The homologous chromosomes then fall apart and parts appear to repel their counterparts and the structure appears as a pair of chromatids. Each chromosome becomes two chromatids. The chromosomes join at several points or crosses known as *chiasmata*. Genetic crossing-over results. Nevertheless, the pair of chromosomes hold until anaphase.

Prophase I is subdivided into different stages:



***Leptotene*:** chromosomes condense, the axial element of the synaptonemal complex (a protein lattice that keeps homologous chromosomes together) is formed. The thin chromosomal threads shorten and become thick so that they are visible as individual threads. One end of each thread is attached to the nuclear membrane. Each thread shows a beaded appearance throughout their length known as chromomeres. The separate chromatoids can not be seen.

More on Synaptonemal Complex: A protein structure that forms between two homologous chromosomes during meiosis and that is thought to mediate chromosome pairing, synapsis, and recombination. The synaptonemal complex is a tripartite structure consisting of two parallel lateral regions and a central element. Three specific components of the synaptonemal complex have been characterized: SC protein-1 (SYCP1), SC protein-2 (SYCP2), and SC protein-3 (SYCP3). The SYCP1 gene is on chromosome 1p13; the SYCP2 gene is on chromosome 20q13.33; and the gene for SYCP3 is on chromosome 12q.

***Zygotene*:** The synaptonemal complex is formed (central and lateral elements) pairing homologous chromosomes, **1% of DNA that wasn't replicated during the S phase replicates**. The two members of a homologous pair of chromosome come to lie side by side forming a bivalent. This pairing is point to point so that the same regions of the chromosome lie in contact. The process is called **synapsis** or conjugation. In the case of human males, the X and Y pair only in limited segments known as pairing segments. The remaining regions are differential segments. The entire pairing is stabilized by a synaptonemal complex in the form of Ribonucleo- proteinaceous fibrillar band occupying a space of about 100 nm.

***Pachytene*:** starts right after the synaptonemal complex is fully formed (when synapsis ends), crossing over occurs. More shortening and thickening of the chromosomes occurs so that now the two chromatids joining at centromere is clearly visible. Thus, each pair of chromosome consists of four chromatids called as **tetrad**.

The important event of recombination, the **crossing over** [C.O.] now takes place or recombination or chiasma where exchange of genetic material between the two homologous chromosomes take place. The molecular mechanism of crossing over is now clearly understood. A large number of proteins which form the synaptonemal complex participate in the process. C.O. leads to a large variety of the final genetic

makeup of an individual. Precise timing and nature of DNA exchange is uncertain and it occurs even in the next stage of Diplotene as suggested by many recent evidences from molecular biological studies[Herris et al 2003] .

***Diplotene*:** synaptonemal complex dissasembles (homologous chromoses are kept together by recombination nodules), there is cellular growth and genetic transcription (and so chromosomes are seen less condensed). Now the homologous chromosomes move away to separate except at chiasmata points.

Later in this phase, the Chiasmata breaks. Normally, at least one chiasmata is formed between each pair but up to even five have been observed. Sometimes the chiasmata move towards the end of chromatids.

***Diakinesis*:** chromosomes condense again, Chiasmata (visual evidence of crossing over) can be seen. The remaining chiasma finally break separating the two chromatids. The bivalents now move away from each other and spread against the nuclear membrane. The nucleoli disappear. Spindle and aster form as in mitosis.

The end of prophase is characterized by the disappearance of nuclear membrane and bivalent chromosomes moving towards the equatorial plane of cell.

In the human ovary, oocytes are stored in the diplotene stage since fetal life. Only just prior to ovulation meiosis I is resumed and is then completed at the time of ovulation (meiosis II ends only just after fertilization). Oocytes that are not ovulated do not complete meiosis I.

The end of Prophase I (also known as **Prometaphase I**) is signified by contraction and staining of all chromosomes, as well as the polar migration of centrioles, the dispersion of the nucleoli and nuclear envelope and the formation of the spindle fibres (including their attachment to chromosomes).

Metaphase 1

In **Metaphase I**, the nuclear envelope has already dispersed into vesicles. Spindle fibres (microtubules) attach only to one kinetochore of both whole centromeres, lining up the bivalents along the equator so that each centromere is equidistant above and below the equator. The lining along the equator is random, maternal or paternal homologues may point to either pole, this is known as independent assortment. It is similar to metaphase of mitosis. The difference being only that it is the homologous pair of chromosomes which lie parallel on the equator of the spindle of microtubules with one member on either side of equator.

Thus, *in Meiosis 1 the homologous chromosomes separate from each other, and not the chromatids of replicated chromosomes.*

Anaphase 1

It differs from anaphase of mitosis in that the *centromere does not split.*

Therefore, one whole chromosome of homologous pair move apart to reach the opposite poles of cell. This result in haploid number of chromosomes in each daughter cell. In terms of DNA content, the cell is in Duplicated Haploid stage.

Telophase 1

As there is random positioning of maternal and paternal bivalent chromosomes there is random assortment of maternal and paternal chromosomes in each daughter cell produced by cytoplasmic division during telophase. The first meiotic division effectively ends when the centromeres arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. **Telophase I** includes the disappearance of spindles and spindle fibres as well as the uncoiling of the chromatids and the reformation of the nuclear membrane. Pinching of the cytoplasm or the formation of cell walls occurs, as in mitosis. Cytokinesis may occur. Note that many plants simply skip telophase I and interphase II, going immediately into prophase II. **Interkinesis** (or **Interphase II**) sometimes occurs in animal cells and presents no DNA replication.

Two daughter cells are formed by the end of Telophase I.

MEIOSIS II

There is a brief interval in the form of unique interphase during which there is no DNA synthesis. Otherwise, there is no difference from mitosis, except that the two separating chromatids during anaphase are genetically dissimilar because of Crossing Over that occurred during Prophase I.

Prophase II takes an inversely proportional time compared to telophase I. In this prophase we see the disappearance of the nucleoli and the nuclear envelope again as well as the shortening and thickening of the chromatids. Centrioles move to the polar regions and are arranged by spindle fibres. This arrangement is rotated by 90 degrees when compared to meiosis I.

In **Metaphase II**, the centromeres behave as two entities, organising fibres on each side to both poles aligning on the equator. This is followed by **Anaphase II**, where the centromeres divide and the fibres pull the now separated chromatids (i.e. chromosomes) are pulled behind the centromere.

The process ends with **Telophase II**, which is similar to that found at mitosis. Uncoiling, lengthening and disappearance of the chromosomes occur as well as the disappearance of the fibres and the replication of the centrioles. Nuclear envelopes reform; cleavage or cell wall formation eventually produces a total of four daughter cells, each with an haploid set of chromosomes.

Errors in Meiosis

It is estimated that from 10–20% of all human fertilized eggs contain chromosome abnormalities, and these are the most common cause of pregnancy failure (35% of the cases).

These chromosome abnormalities:

- arise from errors in meiosis, usually meiosis I;
- occur more often (90%) during egg formation than during sperm formation;
- become more frequent as a woman ages.
- **Aneuploidy** — the gain or loss of whole chromosomes — is the most common chromosome abnormality. It is caused by **nondisjunction**, the failure of chromosomes to correctly separate:

- homologues during meiosis I or
- sister chromatids during meiosis II

- Zygotes missing one chromosome ("monosomy") cannot develop to birth (except for females with a single X chromosome).
- Three of the same chromosome ("trisomy") is also lethal except for chromosomes 13, 18, and 21 (trisomy 21 is the cause of Down syndrome).
- Three or more X chromosomes are viable because all but one of them are inactivated.

Significance of Meiosis

If meiosis did not occur, fusion of the gametes would not result in a diploid condition (2n) but 4n. Meiosis also provides opportunities for new combination as well as DNA Repair (through crossing over) of genes, ensuring heritable variation. The reduction of chromosomes from the diploid to the haploid condition separates alleles so that each gamete carries a sole allele for a gene locus. In addition, the orientation of the metaphase I/II equatorial lining-up is random, resulting in new allelic recombinationis. Independent assortment forms the basis of Mendel's second law. Lastly, chiasmata causes genetic breaks and the establishment of new ones.

Difference between Mitosis and Meiosis

Mitosis	Meiosis
1. It occurs in both asexually and sexually reproducing organisms.	1. Meiosis occurs only in sexually reproducing organisms.
2. Mitosis takes place in the somatic cells.	2. It occurs either in the reproductive cells or at the time of germination of zygote or zygospore.
3. It continues throughout the life of a multicellular organism.	3. Meiosis occurs only during sexual reproduction.
4. It is comparatively simple.	4. It is comparatively complicated.
5. Mitosis takes les time to complete.	5. It is takes longer time to complete.
6. It does not introduce variations.	6. It introduces variations.
7. The cells undergoing mitosis may be haploid or diploid.	7. The cells undergoing meiosis are always diploid.
8. It is a single division which produces two cells.	8. Meiosis is a double division. It gives rise to four cells.
9. A cell can undergo repeated mitosis.	9. Meiosis occurs only once.
10. Subsequent mitotic divisions are similar to the earlier ones.	10. The two divisions of meiosis are not similar. The first one is heterotypic or reductional while the second one is homotypic or equational like mitosis.
11. Each chromosome replicates in the interphase before every division.	11. The chromosomes replicate only once, prior to the two divisions of meiosis.
12. The number of chromosomes remains the same after mitosis.	12. The number of chromosomes is reduced to one half after meiosis.
13. The daughter nuclei or cells formed after mitosis are exactly similar to the parent one.	13. The daughter nuclei or cells formed after meiosis are neither similar to the parent one nor to one another.
14. Mitosis takes part in healing, repair and multiplication of cells.	14. Meiosis takes part in the formation of gametophytes or gametes, maintenance of chromosome number of the race and introduction of variations.
15. The whole of DNA replicates in S-phase.	15. 0.3% of DNA replicates in zygotene while 99.7% docs so in S-phase.
16. Chromomeres are not conspicuous.	16. Chromomeres are quite conspicuous.
17. Prophase is of shorter duration.	
18. Prophase is simpler.	
19. It is loosely distinguishable into 3 substages.	

20. No bouquet stage is recorded.	17. Prophase I is of longer duration while prophase II is very brief. 18. Prophase I is complicated. Prophase II is, however, very simple. 19. It has 5 well distinguishable substages.]
21. Pairing of chromosomes does not occur in mitosis.	20. Chromosomes of animals and some plants show convergence towards one side during early prophase I. It is known as bouquet stage.
22. A synaptinemal complex is absent.	21. Pairing or synapsis of homologous chromosomes takes place during zygotene of prophase I and continues upto metaphase I.
23. Crossing over is absent.	22. Synapsed homologous chromosomes develop synaptinemal complex. 23. Crossing over or exchange of similar segments between nonsister chromatids of homologous chromosomes usually takes place during pachytene diplotene stage.
24. Chiasmata are absent.	24. Chiasmata or visible connections between homologous chromosomes or bivalents are observed during diplotene, diakinesis (prophase I) and metaphase I.
	25. Active transcription may occur in oocytes during diplotene stage.
25. Active transcription is absent.	26. Chromatids become clear only during diplotene of prophase I.
26. The chromatids of the different chromosomes become clear very early in the prophase.	27. A double metaphasic plate is formed by centromeres in metaphase I but only one in metaphse II.
27. Centromeres produce a single metaphasic plate.	28. Homologous chromosomes form bivalents.
28. Chromosomes are free from one another.	29. Chiasmata an present in metaphase I. They are absent in metaphase II.
29. Chiasmta are absent.	30. Centromeres project towards the poles.Limbs of the chromosomes mostly lie over the equator.
30. Centromeres lie over the equator.	31. Limbs of the chromosomes mostly lie over the equator.
31. Limbs of the chromosomes are oriented in different direction.	32. A centromere is connected to only one spindle pole in metaphase I but both in metaphase II.
32. A centromere is connected with both the spindle poles.	33. The two chromatids of a chromosome are often genetically different due to crossing over.
33. Two chromatids of a chromosome are genetically similar.	34. There is no separation of chromatids in anaphase I but chromatids do separate in anaphse II.
34. Chromatids separate to produce daughter chromosomes.	35. Homologous chromosomes separate during anaphse I.
35. There is no separtion of homologous chromosomes.	36. The shorter chromosomes separate earlier than the longer chromosomes during anaphse I.
36. The daughter chromosomes separate almost simultaneously during anaphase.	37. Centromeres do not divide during anaphase I but do so in anaphase II.
37. A centromere splits lengthwise to form two centromeres in the beginning of anaphase.	38. Chromosomes are double stranded in anaphse I but single stranded in anaphse II.
38. Anphsic chromosomes are single stranded.	39. Dissimilar chromosomes move towards the opposite poles in anaphse II.
39. Similar chromosomes move towards the opposite poles in anaphase.	40. Telophase I is shorter and nuclei never enter the interphase.
40. Telophase is longer and produces interphase nuclei.	41. Cytokinesis often does not occur after the first or reductional division. It is then simultaneous after the second division to result in four new cells.
41. Cytokinesis follows every mitosis so that two new cells are formed.	

CELL SIGNALLING, CELL RECEPTORS AND SIGNAL TRANSDUCTION

Introduction to Cell Signaling

In multicellular organisms, there is a need for the cells to communicate with one another in order to coordinate their growth and metabolism. This coordination not only enables the organisms to respond to the external environment but also to the intrinsic genetic programming. There are two principle ways, in which the cells communicate with one another.

1. Neural Communication, i.e. communication involving nerve impulses which are responsible for quick but mostly short term effects.
2. Chemical Communication (also called as Signaling Communication), in which two cells communicate with each other with chemical signaling agents. Here, the responses are slower in comparison to the neural communication but mostly the effects are longer lasting. *Quite often, organisms also respond to chemical signals or signals in the form of some type of energy which are external in origin (that is, synthesized outside the individual's body). Such signals also bring about some major changes in the physiology or behaviour of the organism.*

In the terminology of molecular cell biology hormones are synonymous to *secretable signaling molecules*.

Definition statement

Cell signaling can be defined as a process whereby a chemical substance or energy form (such as light etc.) Acts *in relatively small quantities* upon a target cell through a specific receptor, usually initiating a cascading series of biochemical events which ultimately bring about profound, yet predictable physiological or behavioural changes. The signaling agent never acts as a nutrient, metabolite or as a catalyst.

The most frequent ways in which cellular physiology can be altered by cell signaling include the following.

1. The pattern of gene expression may get altered, i.e. the expression of a particular gene may be stimulated or repressed. This is achieved in any one of the following ways:
   a. activation or repression of a transcription factor
   b. activation or removal of a gene repressor
   c. activation of the gene encoding a relevant transcription factor
   d. kinase cascade mediated gene expression stimulation
   e. formation of a mediator complex

   A change in gene expression pattern of a cell brings about the most far reaching and long lasting changes in the cell including cellular differentiation. Therefore, cell biologists call this

effect as the *Long Term Effect of Signaling*. Rest other effects are grouped under the *Short term Effects of Cell Signaling* even though some of them can be quite stable.

2. The decision to undergo cellular division may be taken or delayed. The chemical signals which stimulate the process of cell division are called *Mitogens*.
3. The activity of cellular enzymes may be enhanced or repressed.
4. The activity of cellular transporters may be enhanced or repressed.
5. The activity or organization of cytoskeletal elements may be altered, which bring about a change in cell shape and locomotary properties
6. the attachment of the cell with other cells in the tissue can be changed resulting into epithelial to mesenchymal transition or vice-versa.
7. The apoptotic process can be triggered by cell signaling process including the *Fas* Signaling System or certain other similar signaling system.
8. The decision to undergo mating (in unicellular forms, such as *Saccharomyces*) can be stimulated.

Components and design of a signaling system

Our current understanding signaling pathways is based on the pioneering work of Earl W. Sutherland (Nobel Prize in 1971). Sutherland and his colleagues at Vanderbilt University were investigating how the animal hormone epinephrine stimulates the breakdown of the storage polysaccharide glycogen within liver cells and skeletal muscle cells.

Sutherland's work suggested that the process of hormonal stimulation can be divided between two players.

1. The source cell or the signaling cell, which generates the chemical signal
2. The target cell, which responds to the signal. The target cell has got:
    a. A *specific receptor* for the signaling agent. The receptor binds to the signaling molecule and starts the response process.
    b. A *transducing system* which relays the chemical message from the receptor to the effector pathway in the cell. The transducing system comprises of enzymes and regulatory proteins of the cytoplasm or of the nucleus. This system usually acts in a multistep manner.
    c. An *effector process* which is the last system to be activated in the signaling process and leads to the cellular response.

A signaling process has three stages (Figure 1).

1. Reception
2. Transduction, and
3. Response.

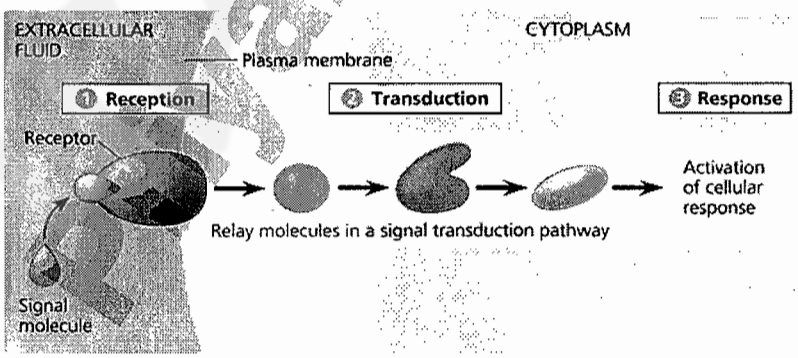


FIGURE 1: THE BASIC STEPS OF SIGNALING PROCESS

Reception. Reception is the target cell's detection of a signal molecule coming from outside the cell. A chemical signal is "detected" when it binds to a receptor protein located at the cell's surface or inside the cell.

Transduction. The binding of the signal molecule

changes the receptor protein in some way, initiating the process of transduction. The transduction stage converts the signal to a form that can bring about a specific cellular response. In Sutherland's system, the binding of epinephrine to a receptor protein in a liver cell's plasma membrane leads to activation of glycogen phosphorylase. Transduction sometimes occurs in a single step but more often requires a sequence of changes in a series of different molecules—a signal transduction pathway. The molecules in the pathway are often called relay molecules. As shown in Figure 2 below, the signal may get greatly amplified during transduction. Due to this, signal transduction is also called *Signal Amplification*. Signal Amplification enables the signaling agents to bring about profound physiological or behavioural changes even while acting in relatively small quantities

Response. In the third stage of cell signaling, the transduced signal finally triggers a specific cellular response. The response may be almost any imaginable cellular activity—such as catalysis by an enzyme (for example, glycogen phosphorylase), rearrangement of the cytoskeleton, or activation of specific genes in the nucleus. The cell–signaling process helps ensure that crucial activities like these occur in the right cells, at the right time, and in proper coordination with the other cells of the organism.

The signal receptors

Signal Substance Receptors are always proteins. Depending on the class of the hormone, the receptor can be located in the Cytosol, Nucleus (for Class I Hormones, *as described later*) or on the cell surface (for Class II Hormones, *as described later*).

Hydrophilic and some large lipophilic hormones (Polypeptide and protein hormones and the catecholamines) bind to cell-surface receptors. The *cell surface receptors are integral membrane proteins* situated in the plasma membrane that bind the signaling molecule (ligand) with high affinity. *The ligand binds to a specific site on the receptor* in a similar specific way as a substrate binds to an enzyme.

The small molecular weight and lipophilic signaling agents (such as steroid, retinoid, and thyroid hormones) have their receptors in the cytosol or in the nucleus. Such signaling molecules cross the plasma membrane and then bind to the recptor.

All receptors have at least two functional domains.

1. A *recognition domain* binds the hormone ligand
2. A *transduction domain* which generates a signal that couples hormone recognition to some intracellular function.

The binding of a signal molecule to its receptor is achieved by a large number of non-covalent chemical bonds. This type of binding is termed *cognate binding*. This is made possible by the presence of a receptor site, which contains many amino acid residues in proper orientation, so that the bonds are easily formed. When a receptor binds to its signaling molecule, it is called as an occupied receptor. The relationship between the concentration of a ligand and the number of occupied receptors is used to describe the term receptor affinity. So, when a large number of receptors are occupied at a relatively low concentration of the ligand, the receptor is called to have a high affinity for the ligand.

Binding of the ligand to the receptor causes a conformational change in the receptor that initiates a *pre-programmed* sequence of reactions in the target cell (often referred to as signal transduction) leading to a change in cellular function.

Coupling (signal transduction) occurs in two general ways. Polypeptide and protein hormones and the catecholamines bind to receptors located in the plasma membrane and thereby generate a signal that regulates various intracellular functions, often by changing the activity of an enzyme. In contrast, steroid, retinoid, and thyroid hormones interact with intracellular receptors, and it is this ligand-receptor complex that directly provides the signal, generally to specific genes whose rate of transcription is thereby affected.

Types of signal receptors

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
RECEPTORS THAT ARE ION CHANNELS	
Ligand-gated ion channels (7, 13)	*Ligands:* Neurotransmitters (e.g., acetylcholine, glutamate), cGMP, physical stimuli (e.g., touch, stretching), IP_3 (receptor in ER membrane) *Receptors:* Four or five subunits with a homologous segment in each subunit lining the ion channel *Signal transduction:* (1) Localized change in membrane potential due to ion influx, (2) elevation of cytosolic Ca^{2+}

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
RECEPTORS LINKED TO TRIMERIC G PROTEINS	
G protein–coupled receptors (13)	*Ligands:* Epinephrine, glucagon, serotonin, vasopressin, ACTH, adenosine, and many others (mammals); odorant molecules, light; mating factors (yeast) *Receptors:* Seven transmembrane α helices; cytosolic domain associated with a membrane-tethered trimeric G protein *Signal transduction:* (1) Second-messenger pathways involving cAMP or IP_3/DAG; (2) linked ion channels; (3) MAP kinase pathway

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
INTRACELLULAR RECEPTORS PATHWAYS	
Nitric oxide pathway (13)	*Ligands:* Nitric oxide (NO) *Receptor:* Cytosolic guanylyl cyclase *Signal transduction:* Generation of cGMP
Nuclear receptor pathways (11)	*Ligands:* Lipophilic molecules including steroid hormones, thyroxine, retinoids, and fatty acids in mammals and ecdysone in *Drosophila* *Receptors:* Highly conserved DNA-binding domain, somewhat conserved hormone-binding domain, and a variable domain; located within nucleus or cytosol *Signal transduction:* Activation of receptor's transcription factor activity by ligand binding

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
RECEPTORS WITH INTRINSIC OR ASSOCIATED ENZYMATIC ACTIVITY	
TGFβ receptors (14, 15)	*Ligands:* Transforming growth factor β superfamily (TGFβ, BMPs), activin, inhibins (mammals); Dpp (*Drosophila*) *Receptors:* Intrinsic protein serine/threonine kinase activity in cytosolic domain (type I and II) *Signal transduction:* Direct activation of cytosolic Smad transcription factors
Cytokine receptors (14, 15)	*Ligands:* Interferons, erythropoietin, growth hormone, some interleukins (IL-2, IL-4), other cytokines *Receptors:* Single transmembrane α helix; conserved multi-β strand fold in extracellular domain; JAK kinase associated with intracellular domain *Signal transduction:* (1) Direct activation of cytosolic STAT transcription factors; (2) PI-3 kinase pathway; (3) IP_3/DAG pathway; (4) Ras-MAP kinase pathway
Receptor tyrosine kinases (14)	*Ligands:* Insulin, epidermal growth factor (EGF), fibroblast growth factor (FGF), neurotrophins, other growth factors *Receptor:* Single transmembrane α helix; intrinsic protein tyrosine kinase activity in cytosolic domain *Signal transduction:* (1) Ras–MAP kinase pathway; (2) IP_3/DAG pathway; (3) PI-3 kinase pathway
Receptor guanylyl cyclases (13)	*Ligands:* Atrial natriuretic factor and related peptide hormones *Receptor:* Single transmembrane α helix; intrinsic guanylate cyclase activity in cytosolic domain *Signal transduction:* Generation of cGMP
Receptor phosphotyrosine phosphatases	*Ligands:* Pleiotrophins, other protein hormones *Receptors:* Intrinsic phosphotyrosine phosphatase activity in cytosolic domain inhibited by ligand binding *Signal transduction:* Hydrolysis of activating phosphotyrosine residue on cytosolic protein tyrosine kinases
T-cell receptors	*Ligands:* Small peptides associated with major histocompatability (MHC) proteins in the plasma membrane of macrophages and other antigen-presenting cells *Receptors:* Single transmembrane α helix; several protein kinases associated with cytosolic domain; found only on T lymphocytes *Signal transduction:* (1) Activation of cytosolic protein tyrosine kinases; (2) PI-3 kinase pathway; (3) IP_3/DAG pathway; (4) Ras–MAP kinase pathway

TABLE 14-1 Overview of Major Receptor Classes and Signaling Pathways

Receptor Class/Pathway*	Distinguishing Characteristics
PATHWAYS INVOLVING PROTEOLYSIS	
Wnt pathway (15)	*Ligands:* Secreted Wnt (mammals); Wg (*Drosophila*) *Receptors:* Frizzled (Fz) with seven transmembrane α helices; associated membrane-bound LDL receptor–related protein (Lrp) required for receptor activity *Signal transduction:* Assembly of multiprotein complex at membrane that inhibits the proteasome-mediated proteolysis of cytosolic β-catenin transcription factor, resulting in its accumulation
Hedgehog (Hh) pathway (15)	*Ligands:* Cell-tethered Hedgehog *Receptors:* Binding of Hh to Patched (Ptc), which has 12 transmembrane α helices; activation of signaling from Smoothened (Smo), with 7 transmembrane α helices *Signal transduction:* Proteolytic release of a transcriptional activator from multiprotein complex in the cytosol
Notch/Delta pathway (14, 15)	*Ligands:* Membrane-bound Delta or Serrate protein *Receptors:* Extracellular subunit of Notch receptor noncovalently associated with transmembrane-cytosolic subunit *Signal transduction:* Intramembrane proteolytic cleavage of receptor transmembrane domain with release of cytosolic segment that functions as co-activator for nuclear trascription factors
NF-κB pathways (14, 15)	*Ligands:* Tumor necrosis factor α (TNF-α), interleukin 1 (mammals); Spätzle (*Drosophila*) *Receptors:* Various in mammals; Toll and Toll-like receptors in *Drosophila* *Signal transduction:* Phosphorylation-dependent degradation of inhibitor protein with release of active NF-κB transcription factor (Dorsal in *Drosophila*) in the cytosol

Signaling molecules

Many different kinds of molecules transmit information between the cells of multicellular organisms. Although all these molecules act as ligands that bind to receptors present in their target cells, there is considerable variation in the structure and function of the different types of molecules that serve as signaling agents.

The salient features of signaling molecules are as follows.

1. All these molecules act as ligands that bind to receptors present in their target cells.
2. There is considerable variation in the structure and function of the different types of molecules that serve as signaling agents. Structurally, the signaling molecules used by plants and animals range in complexity from simple gases to proteins.
3. Some of these molecules carry signals over long distances, whereas others act locally to convey information between neighboring cells.
4. Signaling molecules differ in their mode of action on their target cells. Some signaling molecules are able to cross the plasma membrane and bind to intracellular receptors in the cytoplasm or nucleus, whereas most bind to receptors expressed on the target cell surface.

Signaling molecules can be classified according to chemical composition, solubility properties, location of receptors, and the nature of the signal used to mediate hormonal action within the cell. A classification based on the last two properties is illustrated in the table below.

General Features of Hormone Classes.		
	Group I	**Group II**
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma half-life	Long (hours to days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	cAMP, cGMP, Ca^{2+}, metabolites of complex phosphoinositols, kinase cascades

As it is clear from the table that the hormones in group I are lipophilic. After secretion, these hormones associate with plasma transport or carrier proteins, a process that circumvents the problem of solubility while prolonging the plasma half-life of the hormone. The relative percentages of bound and free hormone are determined by the binding affinity and binding capacity of the transport protein. The free hormone, which is the biologically active form, readily traverses the lipophilic plasma membrane of all cells and encounters receptors in either the cytosol or nucleus of target cells. The ligand-receptor complex is assumed to be the intracellular messenger in this group.

The second major group consists of water-soluble hormones that bind to the plasma membrane of the target cell. Hormones that bind to the surfaces of cells communicate with intracellular metabolic processes through intermediary molecules called second messengers (the hormone itself is the first messenger), which are generated as a consequence of the ligand-receptor interaction.

The important classes of signaling molecules are described below.

1. **Steroid Hormones:** The steroid hormones (including testosterone, estrogen, progesterone, the corticosteroids, and ecdysone) are all synthesized from cholesterol. Testosterone, estrogen, and progesterone are the sex steroids, which are produced by the gonads. The corticosteroids are produced by the adrenal gland. They include the **glucocorticoids**, which act on a variety of cells to stimulate production of glucose, and the **mineralocorticoids**, which act on the kidney to regulate salt and water balance. Ecdysone is an insect hormone that plays a key role in development by triggering the metamorphosis of larvae to adults.
2. **Thyroid hormones, vitamin D_3, and retinoic acid:** Although thyroid hormone, vitamin D_3, and retinoic acid are both structurally and functionally distinct from the steroids, they share a common mechanism of action in their target cells. Thyroid hormone is synthesized from tyrosine in the thyroid gland; it plays important roles in development and regulation of metabolism. **Vitamin D_3** regulates Ca^{2+} metabolism and bone growth. **Retinoic acid** and related compounds (**retinoids**) synthesized from vitamin A play important roles in vertebrate development.

   Because of their hydrophobic character, the steroid hormones, thyroid hormone, vitamin D_3, and retinoic acid are able to enter cells by diffusing across the plasma membrane. Once inside the cell, they bind to intracellular receptors that are expressed by the hormonally responsive target cells. These receptors, which are members of a family of proteins known as the steroid receptor superfamily, *are transcription factors that contain related domains for ligand binding, DNA binding, and transcriptional activation. Ligand binding regulates their function as activators*

or repressors of their target genes, so the steroid hormones and related molecules directly regulate gene expression.

3. **Nitric Oxide and Carbon Monoxide:** The simple gas nitric oxide (NO) is a major paracrine signaling molecule in the nervous, immune, and circulatory systems. Like the steroid hormones, NO is able to diffuse directly across the plasma membrane of its target cells. The molecular basis of NO action, however, is distinct from that of steroid action; rather than binding to a receptor that regulates transcription, NO alters the activity of intracellular target enzymes.

   Nitric oxide is synthesized from the amino acid arginine by the enzyme nitric oxide synthase. Once synthesized, NO diffuses out of the cell and can act locally to affect nearby cells.

   Another simple gas, carbon monoxide (CO), also functions as a signaling molecule in the nervous system. CO is closely related to NO and appears to act similarly as a neurotransmitter and mediator of blood vessel dilation. The synthesis of CO in brain cells, like that of NO, is stimulated by neurotransmitters. In addition, CO can stimulate guanylate cyclase, which may also represent the major physiological target of CO signaling.

4. **Neurotransmitters:** The neurotransmitters carry signals between neurons or from neurons to other types of target cells (such as muscle cells). They are a diverse group of small hydrophilic molecules including acetylcholine, dopamine, epinephrine (adrenaline), serotonin, histamine, glutamate, glycine, and γ-aminobutyric acid (GABA). The release of neurotransmitters is signaled by the arrival of an action potential at the terminus of a neuron. The neurotransmitters then diffuse across the synaptic cleft and bind to receptors on the target cell surface. Some neurotransmitters can also act as hormones. For example, epinephrine functions both as a neurotransmitter and as a hormone produced by the adrenal gland to signal glycogen breakdown in muscle cells.

   Because the neurotransmitters are hydrophilic molecules, they are unable to cross the plasma membrane of their target cells. Therefore, in contrast to steroid hormones and NO or CO, the neurotransmitters act by binding to cell surface receptors. Many neurotransmitter receptors are ligand-gated ion channels, such as the acetylcholine. Neurotransmitter binding to these receptors induces a conformational change that opens ion channels, directly resulting in changes in ion flux in the target cell. Other neurotransmitter receptors are coupled to G proteins—a major group of signaltransducers that link cell surface receptors to a variety of intracellular responses. In the case of neurotransmitter receptors, the associated G proteins frequently act to indirectly regulate ion channel activity.

5. **Peptide Hormones and Growth Factors:** The widest variety of signaling molecules in animals are peptides, ranging in size from only a few to more than a hundred amino acids. This group of signaling molecules includes peptide hormones, neuropeptides, and a diverse array of polypeptide growth factors. Well-known examples of **peptide hormones** include insulin, glucagon, and the hormones produced by the pituitary gland (growth hormone, follicle-stimulating hormone, prolactin, and others).

   Neuropeptides are secreted by some neurons instead of the small-molecule neurotransmitters. Some of these peptides, such as the **enkephalins** and **endorphins**, function not only as neurotransmitters at synapses but also as **neurohormones** that act on distant cells. The enkephalins and endorphins have been widely studied because of their activity as natural analgesics that decrease pain responses in the central nervous system. Discovered during studies

of drug addiction, they are naturally occurring compounds that bind to the same receptors on the surface of brain cells as morphine does.

The **polypeptide** growth factors include a wide variety of signaling molecules that control animal cell growth and differentiation. The first of these factors (**nerve growth factor**, or **NGF**) was discovered by *Rita Levi-Montalcini in the 1950s*. NGF is a member of a family of polypeptides (called **neurotrophins**) that regulate the development and survival of neurons. During the course of experiments on NGF, Stanley Cohen discovered an unrelated factor (called epidermal growth factor, or **EGF**) that stimulates cell proliferation. EGF is a 53-amino-acid and quite similar to growth factors that play critical roles in controlling animal cell proliferation, both during embryonic development and in adult organisms. Peptide hormones, neuropeptides, and growth factors are unable to cross the plasma membrane of their target cells, so they act by binding to cell surface receptors.

6. **Eicosanoids:** Several types of lipids serve as signaling molecules that, in contrast to the steroid hormones, act by binding to cell surface receptors. The most important of these molecules are members of a class of lipids called the eicosanoids, which includes **prostaglandins**, **prostacyclin**, **thromboxanes**, and **leukotrienes**. The eicosanoids are rapidly broken down and therefore act locally in autocrine or paracrine signaling pathways. They stimulate a variety of responses in their target cells, including blood platelet aggregation, inflammation, and smooth-muscle contraction. All eicosanoids are synthesized from arachidonic acid, which is formed from phospholipids.

Types of signaling processes

In every type of cell communication, there is always a source cell (that generates the signal) and a target cell (which is subjected to signals). Cell communication is often typified **on the basis of the spatial relation** that the source and target cells share.

Accordingly the following major types of cell communication are identified.

1. In **endocrine signaling**, the signaling molecule (e.g. insulin) acts on target cells distant from its site of synthesis in cells of an endocrine organ (e.g. the pancreas). The endocrine cells secrete the signaling molecule into the bloodstream (if an animal) or the sap (if a plant) which carries it to the target cells elsewhere in the organism. In older literature, it was also called Hormonal Signaling – however, in modern usage the term hormonal signaling has a broader meaning, as already described.

2. In **paracrine signaling**, the signaling molecule affects only target cells close to the cell from which it was secreted. The communication from one nerve cell to another by chemical neuro-transmitters is an example of paracrine signaling. During the past decade, developmental biologists have discovered that the induction of numerous organs is actually effected by a relatively small set of paracrine factors. Many of these paracrine factors can be grouped into four major families on the basis of their structures. These families are the fibroblast growth factor (FGF) family, the Hedgehog family, the Wingless (Wnt) family, and the TGF-β superfamily. *Synaptic signaling is also a type of local signaling* in which the signal substance (i.e. Neurotransmitter) is released in a close range.

3. In **autocrine signaling**, a cell responds to a molecule that it has produced itself or a cell of its own type. The best studies example of autocrine signaling in the plant kingdom is the action of Auxins on the apical meristematic zone cells and Ethylene in mature tissues and ripening fruits. In animals, autocrine signaling is essential to bring about the community effect of development during embryonic

differentiation. It is seen in *placental cytotrophoblast cells* which secrete *platlet derived growth factor* for their own fast proliferation.

4. In **Juxtacrine Signaling** (or **Contact Signaling**) the source and target cells are physically in contact as it happents in case of the *Notch* signaling during embryonic development in animals or in case of *Osmotins* and *Systemins* in plants.

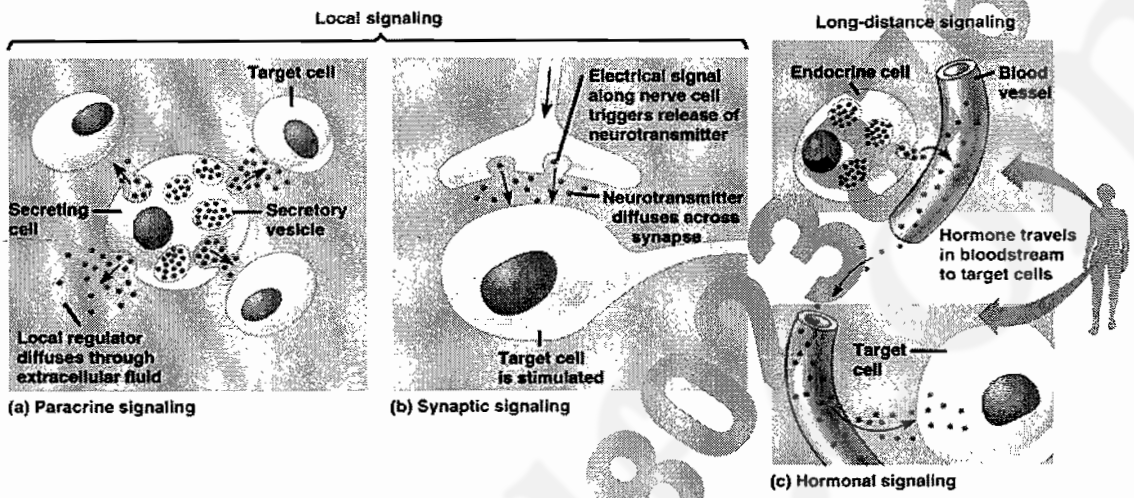


FIGURE 2: LOCAL AND LONG DISTANCE SIGNALING

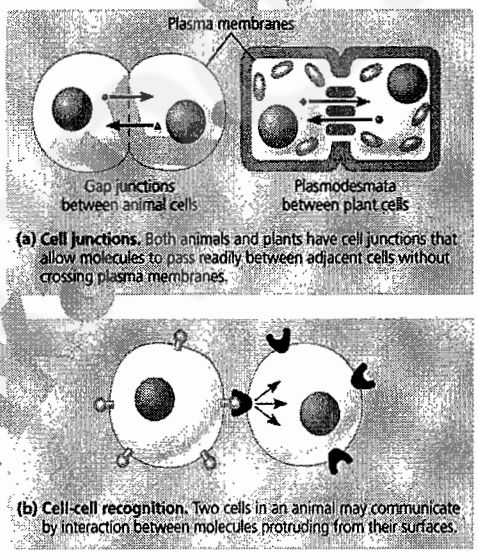


FIGURE 3: CONTACT SIGNALING